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This, the final report for all clinical research studies conducted under the US Army BAA for Neurofibromatosis type 1, reviews the progress made between January, 1994 (Army approved start date) and October, 1997 (completion of data accrual for all studies) concerning the conduct and preliminary results from NF-1 Clinical Trials Consortium studies. It is organized into three sections: (1) a description of the clinical trials structure and organization; (2) a review of the randomized phase II clinical trial results for the treatment of NF-1 patient with optic pathway / hypothalamic gliomas or plexiform neurofibromas; (3) a review of the methods development and results for multimodality neuroimaging studies in NF-1 patients with newly-diagnosed or progressive optic pathway / hypothalamic gliomas or brain stem gliomas.

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Peter C. Phillips, M.D. October 27, 1997

PI - Signature Date

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INTRODUCTION

Patients with Neurofibromatosis Type 1 (NF1) are at high risk for the development of potentially life-threatening intracranial or systemic tumors. Fifteen to 20% of NF-1 patients have Optic Pathway tumors, 5% have brainstem masses, and despite advances in diagnosis and treatment, these histologically benign tumors often have a clinically malignant outcome. Furthermore, 50 % of NF-1 patients will have at least one peripheral plexiform neurofibroma and nearly one third of these patients will have severe disabilities or life-threatening dysfunction directly attributable to their plexiform neurofibromas.

Advances in the treatment of intracranial and peripheral tumors in NF-1 patients have been impeded by several problems. First, the rate of tumor growth is extremely variable between different patients and even within the same patients. Periods of spontaneous growth arrest after an interval of rapid tumor growth are well-described for NF-1 optic pathway and hypothalamic gliomas. Therefore, it may be difficult to determine when to treat NF-1 patients for tumor progression and it may be even more difficult to determine if tumor growth arrest is attributable to a therapeutic intervention or spontaneous. There are no reliable non-invasive diagnostic modalities that distinguish optic pathway and hypothalamic gliomas with a low growth potential from those with a high growth potential. Second, current treatment options for NF-1 tumors, including radiation therapy and cytotoxic chemotherapy, are often ineffective and may expose NF-1 patients to high risks of treatment-associated second malignancies. Third, current measures of treatment response are based on models of malignant disease that may be inappropriate or inaccurate for these histologically benign masses. Whereas reduction of tumor volume after specific therapy represents an important goal, it is likely that other parameters of treatment response which address biochemical and functional changes in the tumor will have important prognostic value in the assessment of treatment response. This consideration may be particularly important for biological treatments that induce tumor differentiation; that is, the induction of tumor differentiation may lead to growth arrest without volume reduction.

To address these problems, we conducted multimodality neuroimaging studies in NF-1 patients with newly-diagnosed or progressive optic nerve / hypothalamic or brain stem tumors to predict the growth potential of these tumor and a randomized clinical trial of new antitumor agents in progressively enlarging OPT/HT and plexiform neurofibromas to rapidly identify potentially effective therapies. The three year period of funding was not sufficient for us to complete accrual and obtain sufficient follow-up studies in the various patient populations. Therefore, the US Army granted permission for an unfunded extension of our studies beyond the 10/96 termination date. On January 10, 1997, we submitted a three year progress report which summarized the activities of our clinical research program from February through December, 1996. That report was accepted by the review committee with the understanding that we would submit an amended l report in October, 1997 to include data and conclusions obtained during the interval 10 months.

This, the revised final report for all clinical research studies conducted under the US Army BAA for Neurofibromatosis type 1, reviews the progress made between January, 1994 (Army approved start date) and October, 1997 (completion of data accrual for all studies) concerning the conduct and results from NF-1 Clinical Trials Consortium studies. It is organized into three

sections: (1) a description of the clinical trials structure and organization; (2) a review of the randomized phase II clinical trial results for the treatment of NF-1 patient with optic pathway / hypothalamic gliomas or plexiform neurofibromas; (3) a review of the methods development and results for multimodality neuroimaging studies in NF-1 patients with newly-diagnosed or progressive optic pathway / hypothalamic gliomas or brain stem gliomas.

I. NF-1 CLINICAL TRIALS ORGANIZATION

A. Advisory Committees

To facilitate recommendations made by the Special Review Committee a Neurofibromatosis Research Steering Committee was impaneled on June 21,1995. Steering Committee's responsibilities include: (1) development of standard methods and procedures for the conduct of all aspects of research conducted in this study; (2) formal review of the conduct of all aspects of research conducted in this study with respect to newly-established timelines and research goals; (3) identification of existing or emerging problems in research study conduct or design and the development alternative solutions to these problems; (4) formal, open review of data analysis data interpretation, and preliminary conclusions for all aspects of this research study; and (5) presentation to the External Advisory Committee of all research communications intended for the medical scientific community.

The Neurofibromatosis Research Steering Committee is chaired by Dr. Phillips (Grant Pl) and includes the following members: Avital Cnaan, Ph.D., Director of data management and statistical analysis; Patricia Molloy, M.D., co-investigator and project director for NF1 neuroimaging studies; Michael Needle, M.D., co-investigator and project director for NF1 clinical trials; and Sheila Vaughan, R.N., clinical coordinator for NF1 neuroimaging studies and clinical trials. Because of the technical complexity of the neuroimaging studies, a Neuroimaging Advisory Panel was established. This panel also includes Dr. Abass Alavi (Director of Nuclear Medicine at The Hospital for the University of Pennsylvania, and Co-Director of the PENN PET Center), Dr. John Hazelgrove (Director of Research Physics, Division of Neuroradiology at CHOP), and Drs Allison Hoydu and Jerry Wang (Research Physicists for MRI-flow and MRSpectroscopy, respectively, in the Division of Neuroradiology at CHOP. The Neuroimaging Advisory Panel reports directly to the Steering Committee and attends open meetings of the External Advisory Committee. Since its establishment, the Neurofibromatosis Research Steering Committee has met 28 times and the minutes of these meetings have been distributed to the off-site Consortium collaborator.

On July 10,1995, we impaneled a Neurofibromatosis Research External Advisory Committee Nominations for this Committee were made by members of the Steering Committee and selection of External Advisory Committee members was based on the following criteria: (1) all members must have no direct involvement in the conduct of research for this study; (2) Committee members must have recognized expertise in clinical research trial design, the conduct of consortium clinical trials, and/or the design and conduct of neuroimaging research trials; (3) Committee member's availability and willingness to meet frequently with the Steering Committee during the summer months and then continue their advisory role on a quarterly basis thereafter.

The External Advisory Committee is chaired by Dr. Edwin Douglass, Director of Clinical Oncology at the St. Christopher's Hospital for Children, Philadelphia, PA. Dr. Douglass is nationally recognized for his clinical research achievements. In addition, Dr. Douglass has direct and extensive experience in the diagnosis and treatment of childhood brain tumors as a member of the Neuro-Oncology Program at St. Jude Children's Research Hospital. The four additional Committee members are balanced evenly between those with clinical trials experience and those with neuroimaging study experience. Dr. Giulio D'Angio, Professor of Radiation Oncology at the Hospital for the University of Pennsylvania, has an international reputation for his leadership in the National Wilm's Tumor clinical consortium. Dr. James Boyett, Chairman of the Department of Biostatistics at the St. Jude Children's Research Hospital, is nationally recognized for his achievements in statistical analysis and clinical trial design and conduct. He has also served as a biostatistician for the Brain Tumor Strategy Group in the Children's Cancer Group for the past seven years. St. Jude Hospital is not a NF1 clinical consortium member. Dr. Henry Holcomb, Assistant Professor of Psychiatry at the University of Maryland, is nationally recognized for his Positron Emission Tomography studies of cerebral metabolism abnormalities in psychiatric disease.

Dr. William Negendank is a nationally recognized expert in magnetic resonance imaging and magnetic resonance spectroscopy studies of the brain, worked closely with our group as a member of the External Advisory Committee until his untimely death last year. Because all of the methods development had been completed, we did not seek a replacement for Dr. Negandank on the External Advisory Committee

The External Advisory Committee met in open sessions with the Steering Committee members at the Children's Hospital of Philadelphia on a monthly basis from July through October, 1995 and semiannually until January, 1997. Closed meetings of the External Advisory Committee have also been held and additional communication between the Committee members and between the Steering Committee and the External Committee have been conducted by phone and fax. External Advisory Committee Members report directly to the Committee Chairman. Specific Committee responsibilities include: (1) review of the organizational structure of all U.S. Army-sponsored NF1 research activities to assure the independence of data collection/management and data analysis and interpretation; (2) review of research study problems and proposals by the Steering Committee for their solution. Provide specific advice relevant to the solution of those problems; review of the data management input functions, including an assessment of data retrieval, database structure, accuracy of database entry, and completeness of required data entry points; (3) assist the Steering Committee with the process of establishing and monitoring realistic timetables to achieve expected patient accruals, data entry and analysis, and report the conclusions of these studies to the scientific and medical community; (4) review all research communications intended for the scientific and/or medical communities to assure the accuracy of data and validity of conclusions prior to their submission to meetings or for publication and Provide the Program Pi with a critique of proposed research communications and an indication of the of level of enthusiasm for all such research communications.

B. Multi-Institution Clinical Consortium

The consortium, as originally proposed, consisted of The Children's Hospital of Philadelphia as the lead institution and ten collaborating consortium members. Selection of the Consortium institutions was based on three factors: (a) the presence of a large neurofibromatosis clinical referral base; (b) participation by the Consortium institution in a major children's cancer consortium (e.g., the Children's Cancer Group (CCG) or the Pediatric Oncology Group (POG), thereby providing a level of assurance that the institution and the participating investigators were familiar with the procedures and responsibilities of a clinical consortium; and (3) indication by the principal investigators for each Consortium institution that they had at least two patients with progressive growth of optic pathway tumors each year and would be willing to participate in these studies. During the first 18 months of these clinical studies, it became apparent that several institutions who indicated their willingness to participate were not able to do so, either because their institutional IRBs would not accept the requirement specified by the U.S. Army that the local institution accept all financial responsibility for medical complications arising from the conduct of this trial (Chicago and Buffalo) or because of interdepartmental disagreements concerning the priority of this protocol versus other institutional protocols (M.D. Anderson). In response to these problems, we replaced M.D. Anderson with Reilly Children's Hospital (R. Jakacki, M.D., P.I.), University of Chicago with Washington University (D. Guttman, M.D., Ph.D., PI), and Buffalo with University of Arkansas (pending IRB approval; J. Ochs, M.D., P.I.). We notified the U.S. Army of these changes and worked with each institution to assist them with U.S. Army IRB approval. In consultation with our External Advisory Committee, we decided not to significantly increase the size of the existing clinical consortium.

C. Data Management and Security

Dr. Cnaan, Director of Biostatatistics, directly oversees all data management, data entry, correction and summary for the Neurofibromatosis Research studies. The Study Coordinator (Ms. Sheila Vaughan) reports to Dr. Cnaan, the Director of Data Management and Biostatistics, on all issues of data management. Ms. Vaughan establishes patient eligibility by telephone with a physician at an outside institution or with Drs. Molloy or Needle at CHOP. Ms. Vaughan initiates an On-Study form and sends a copy of the complete On-Study Report Form to the referring institution or to Dr. Molloy or Needle in order to confirm the accuracy of the phone contact.

Two databases have been created using Filemaker Pro®, a commercially available database program; one for the chemotherapy clinical trial and a separate database for the neuroimaging study. Because their formats are structurally similar, these databases are able to exchange information for the small number of patients that may participate in the neuroimaging and the chemotherapy clinical trial. Furthermore, the output from these databases are converted easily to crossplatform Excel or ASCII formats; therefore, information contained in this database can be shared with other clinical neurofibromatosis databases, including that of the University of British Columbia. We revised our data collection forms to conform to the database structure. The database contains a "layout" for each form. The forms are: On-Study, Dose, Response, Laboratory, Toxicity, and Off-Study.

We made appropriate provisions for the physical safety of all study data. The data in the database is backed up onto a diskette once a week by Mr. Paul Gallagher, who constructed the database according to Dr. Cnaan's specified design. Mr. Gallagher keeps the backup diskette in his office, while the computer within which the database actually resides, is in a separate building in the Dept. of Neurology. Entry to the database is restricted by password. Currently, only Sheila Vaughan and Paul Gallagher, have access to the database. Dr. Needle has an additional backup of the database in his office, providing a second backup site. He receives a backup diskette from Mr. Gallagher once every three months.

II. RANDOMIZED PHASE II TRIAL OF CIS-RETINOIC ACID, INTERFERON a2A, AND ETOPOSIDE IN NF-1 PATIENTS WITH PROGRESSIVELY ENLARGING OPTIC PATHWAY / HYPOTHALAMIC GLIOMAS OR PLEXIFORM NEUROFIBROMAS.

A. Introduction

Three agents were selected for clinical trial. Oral VP-16, a conventional cytotoxic which has shown activity against low grade gliomas was selected for the optic pathway tumor clinical trial. This stratum of out clinical trial, therefore, involves the treatment of a bona fide neoplasm. However, we did not include oral VP-16 in the treatment randomization of progressive plexiform neurofibromas (i.e. not bona fide neoplasms) due to the potential of this and other conventional chemotherapeutic agents to cause secondary tumors.

Rationale for the use of a2a interferon (IFN) comes from the published studies by Dr. Judah Folkman concerning the anti-angiogenic action of a2a IFN. In this model all tumors, benign or malignant, need a growing vascular supply to support tumor growth. Any agent that will interfere with angiogenesis should inhibit or reverse tumor progression. One agent currently licensed for use in the United States which has these properties is IFN. IFN has been used to treat children with life threatening hemangiomas of infancy resistant to steroids]. IFN has demonstrated activity against meningioma cell lines derived from patients with NF 2 in-vitro], and has direct antitumor activity against hairy cell leukemia. It may also exert some effect on solid tumors apart from any angiogenic activity.

Rationale for the use of cis-retinoic acid (CRA) is based on its potential as differentiating agents in cancer. All-trans retinoic acid is effective in the treatment of acute promyelocytic leukemia. CRA has demonstrated activity in neuroblastoma, although it's role in the management of this tumor remains minimal. CRA is the subject of intense investigation as a chemoprotectant for breast cancer and has been demonstrated to reduce the incidence of tumor recurrence in patients following treatment for aerodigestive tract cancer. Published data suggests that CRA alters the splicing pattern of the NF 1 gene transcript; however, this observation has not been tested directly in PN cell lines or in malignant tumor cell lines from patients with NF 1.

B. Methods

Patient Population:

Patients who were older than 12 months of age, met NIH consensus criteria for the diagnosis of NF-1, and had objective evidence of progressive enlargement of a tumor of the optic nerve, optic chiasm, optic radiations, or hypothalamus (Stratum I), or a disfiguring or disabling plexiform neurofibroma (Stratum II) were eligible for treatment. Patients with recurrent or progressive intracranial tumors that were previously treated with radiation therapy and/or chemotherapy are eligible. Specific exclusions include pregnancy, visual acuity less than 20/200 in one or both eyes, brainstem glioma, histology confirmed diagnosis of malignant glioma (i.e., anaplastic astrocytoma, glioblastoma, or gliosarcoma) or other malignant histology, rapid progressive symptomatic spinal cord compression (PN), or other rapidly progressive life-threatening complications of plexiform neurofibroma growth. Female patients who have reached menarche must have a negative serum b-HCG within 48 hours prior to each therapy cycle. Biopsy confirmation of tumor histology is not required for study entry. Children's Hospital of Philadelphia Institutional Review Board- and US Army-approved Informed Consent Documents were signed by patients and/or parents of patients prior to participation in these studies.

We modified the entry criteria for the Optic Pathway stratum with full approval from the External Advisory Committee in 9/1/95. Accordingly, all optic pathway tumor patients must have documentation of progression either by MRI or by a change in visual acuity of two steps on standard visual acuity charts within six months of study entry. These modification effectively prevent study entry for patients who had an MRI two years ago and the next MRI two months before study entry. In fact, all optic pathway patients currently on study meet these criteria.

Stratum 1 Treatment: Optic Pathway / Hypothalamic Tumor Phase II Trial

At the onset of this study, eligible patients were randomly assigned to one of three treatment arms: Arm 1 - cis retinoic acid (CRA; 60 mg/m2 by mouth daily for 21 days followed by 7 days of no drug treatment x 12 months); alpha 2a Interferon (1,000,000 with dose escalation in increments of 500,000 units to a maximum of 4,000,000 IU/m2/day administered by subcutaneous injection daily for 12 months); or etoposide (VP-16; 50mg/m2, daily by mouth for 21 days followed by 7 days with no drug treatment. Volumetric MRI scans were performed every 12 weeks to assess treatment response and MRS and PET scans were performed at 3 months and 12 months after the start of treatment. Because of poor accrual to Stratum 1, we terminated randomization procedures in order to complete the phase II study of etoposide. This action was taken in October, 1995 at the advice of our External Advisory Committee. Full notification of the US Army was made and approval obtained. Consent forms and IRB documents were modified to reflect these changes.

Stratum 2 Treatment: Plexiform Neurofibroma Phase II Trial.

Eligible patients referred for treatment of progressively disfiguring or disabling plexiform neurofibromas were randomly assigned to one of two treatment arms: Arm-1, 13- Cis Retinoic Acid (CRA); Arm-2, Alpha Interferon 2a (INF). Patients assigned to the CRA treatment arm received a dose of 60mg/m2, daily by mouth for 21 days followed by seven days of no drug treatment. This 28-day treatment cycle is repeated for 13 cycles (1 year). Patients assigned to the Alpha Interferon 2a arm were treated with an initial dose of 1 x 10^6 IU/m2 administered daily by subcutaneous injection for one year. Objective evidence of response was assessed every 12 weeks after the start of treatment, based on direct measurement of surface neurofibromas or soft-tissue MRI scan of deep neurofibromas. For all patients, routine complete blood counts and

blood chemistry values were monitored on a regular basis, weekly during treatment with VP-16 and monthly for treatment with CRA and INF.

The plexiform neurofibroma (PN) strata accrued patients at two times the rate that was originally projected. In joint consultation and with the explicit approval of the External Advisory Committee, we made three changes in the plexiform neurofibroma study. We modified our study objectives to include an assessment of cessation of tumor growth as a treatment outcome. We also tightened patient entry criteria by requiring more rigorous objective evidence of tumor growth (i.e., MRI or recorded tape measurements independently by two different physicians) within no more than six months from data of study entry. In addition, we modified patient accrual targets for plexiform neurofibroma patient entry to allow us to enroll a total of 56 patients who meet the more rigorous documentation criteria for tumor progression prior to study entry. This will allow us to evaluate more "clinical observation" evidence of response, and generate hypotheses regarding cessation of tumor progression as an outcome measure. These modifications do not require a change in the consent form; however, we revised our protocol to indicate the changes, submitted the amended protocol to the CHOP IRB for review, received approval from the CHOP IRB on September 25, 1995, notified the US Army of these changes in research design, and provided an amended protocol to all Consortium members.

All patients in this study were required to have objective evidence of plexiform neurofibroma growth determined by radiologic (e.g. CT or MRI) or direct measurement (e.g. physician tape measurements) criteria. However, at the onset of the trial, we did not specify the interval between measurements. If we use tumor stabilization as a criterion, we must be certain that patients entering study are truly progressing, i.e., have actively growing tumors at study entry. Toward that end, we tightened entry criteria by specifying the objective measure of tumor progression at study entry: that is, serial MRI demonstrating tumor growth within but no longer than the last 6 months or by serial external tape measurements independently by two observers within a six month interval.

C. Results

Stratum I: Optic Pathway / Hypothalamic Glioma Phase II Trial.

As noted above (Methods), our original design was a randomized phase II study between IFN, CRA and etoposide (VP-16). When it became apparent that accrual was insufficient to fill all three arms, a decision was made to enter all subsequent patients on the VP-16 arm. Therefore, 8 patients received VP-16, 2 received CRA, and 3 receive INF.

Total patient accrual to the optic pathway tumor arm of the study was less than originally anticipated. The original design was a randomized phase II study between IFN, CRA and etoposide (VP-16). When it became evident that accrual was not sufficient to fill all three arms, a decision was made to enter all subsequent patients on the VP-16 arm. In total, 8 patients received VP-16, 2 received CRA, and 3 receive INF.

Thirteen patients were enrolled to Stratum I. There were 7 males and 6 females. All patients had at least two of the diagnostic criteria of NF1. They all had optic nerve glioma. 10 met the diagnostic criteria by having greater than 6 café-au-lait macules, one by family history, one by a

characteristic skeletal anomaly. The age ranged from 21 months to 27 years. The median was 5 years and the mean 8.8 with a standard deviation of 8.8 years. All of the patients were Caucasian. Seven of the eight patients on VP-16 are evaluable. One is lost to follow-up.

One patient had a minor response to VP-16 treatment as evidenced by approximately 25% tumor shrinkage . Three patients progressed on therapy. The remainder remained stable for the 12 month duration of the study. Only one patients on VP-16 experienced grade 3 hematologic toxicity. No other grade 3 or 4 toxicity was noted.

Stratum 2: Plexiform Neurofibroma Phase II Trial.

Sixty three patients were entered on Stratum 2. Of these 63 patients 4 signed the consent were registered but did not return for any follow up visit. They are not evaluable for toxicity or efficacy. There were 28 males and 35 females. All patients had at least two of the diagnostic criteria of NF1. They all had plexiform neurofibroma. Fifty four patients met the diagnostic criteria by having greater than 6 café-au-lait macules, 9 by axillary or inguinal freckling, 5 by family history, two by Lisch nodules, and one by optic nerve glioma. The age ranged from 3 months to 61 years. The median age was 11 years and the mean 14 with a standard deviation of 13. Forty six patients were Caucasian, 10 African American, 3 Hispanic, and one Asian. The initial assignment by randomization was 34 patients to the CRA arm and 29 patients to the IFN arm.

Patients were randomized to receive either CRA by oral capsule, or IFN by subcutaneous injection, for one year. Patients were monitored by monthly physical exam, monthly routine blood counts and chemistries, and by cross sectional imaging every 12 weeks. The criterion for a partial response was established as 50% tumor shrinkage. Minor responses were defined as tumor shrinkage of greater than 25% but less than 50%. No patient had tumor shrinkage of this magnitude reported. However, a number of observations suggested that the drugs in question may be having an effect. Of the 30 evaluable patients treated with CRA, 3 had evidence of tumor shrinkage by direct measurement of the superficial component of their tumors. Of the 29 patients on IFN, 3 had evidence of tumor shrinkage by direct measurement, one had resolution of bradycardia secondary to a vagal nerve tumor, one had resolution of orthopnea, and two had relief of pain. Overall, 10 of 59 patients (17%) had some evidence of clinical benefit.

Toxicity was relatively low and manageable with both agents. All patients treated with CRA experienced chelitis and dry skin, which was treatable with emollients. The investigators graded this toxicity as grade 1 in 50%, grade 2 in 10%, grade 3 in 20 %. Although ten patients withdrew from therapy due to discomfort, they did so after six months of participation and can be evaluated for efficacy. Patients treated with interferon had some significant toxicities. 3 patients experienced grade 3 hematologic toxicity; one patient experienced grade 4 and 3 patients grade 3 hepatic toxicity; 2 patients experienced grade 3 neurologic toxicity. Nine patients withdrew from interferon because of the pain associated with daily subcutaneous injection, also at a median interval of 6 months.

Prognostic Factors and Progression Rates for Plexiform Neurofibroma

Stratum 2 was not designed to incorporate a control group which did not receive treatment and the rate of growth for plexiform neurofibromas is not known. Therefore, we undertook a retrospective study of the surgical experience of CHOP to identify the rate of neurofibroma growth after surgery and to identifying factors which would predict the outcome of surgery of plexiform neurofibroma. This study describes the only longitudinal data available for plexiform neurofibroma.

We identified 121 patients who underwent surgical resection of 168 individual tumors at The Children's Hospital of Philadelphia between 1974-1994. The total number of procedures was 302 (mean 1.80 per tumor, range 1-12). For the purpose of data analysis the 168 tumors are treated as individual events, as there is no data in the literature to suggest consistent biologic behavior of multiple tumors within a single patient. Data was collected from a number of sources. Data regarding the demographics of the patients was obtained from either the hospital chart, the outpatients records of the surgical services, or the Neurofibromatosis clinic chart. Data regarding the indication(s) for surgery and the extent of surgical excision was gathered from the operative note. When the primary indication for surgery was cosmetic and in the case of lesions not causing pain or dysfunction, the procedures were considered elective. Other indications were dysfunction, pain, suspicion of cancer in patients known to have NF1, and diagnostic biopsy in cases where the diagnosis of NF 1 was uncertain. Data regarding location of tumor was abstracted from the patient chart. It can often be difficult to distinguish multiple tumors in a specific region from a larger infiltrating tumor. We considered all procedures on a single body region (such as the mediastinum or a single extremity) as if the tumor in the region was a single tumor. For the purpose of analysis of location of tumor as a prognostic variable, tumors were assigned to 3 regions, head/neck/face, extremities, and trunk (including thorax, mediastinum, spine, and viscera) (table 2). For the purpose of this study gross-total resection was defined as complete removal of tumor, near total resection was defined as greater than 90% tumor removal, sub-total resection was defined as greater than 50% but less than 90% tumor removal, and biopsy was defined as less than 50% tumor removal. In all cases extent of surgical excision was determined by the operating surgeon at the time of surgery. Follow-up data regarding duration of tumor control, and surgical morbidity was assessed from outpatient charts and by patient interviews in the NF clinic or by telephone. Progression was defined as the reappearance of a completely excised tumor or the regrowth of a partially excised tumor. Kaplan-Meier curves were calculated and logrank tests were used to compare differences between progression-free survival curves based on age, location, indication, and extent of resection. Cox regression models were used to explore predictive importance of prognostic factors for progression-free survival. Primary data analysis was conducted by using tumors as individual events, and only data concerning the first procedure was included. A confirmatory analysis was carried out using one tumor for each patient, using the patient as an independent unit of analysis and thus not needing to assume lack of consistent biological behavior of tumors within the same patient.

We found that ninety-four of the 168 tumors (56%) did not progress after the first surgical procedure; whereas, 74 tumors progressed after surgery. The median duration of follow-up in this study was 6.8 years and ranged from 2 months to 24.5 years.

For the purpose of identifying prognostic factors, only data concerning the first procedure was evaluated. Fifty of 83 children 10 years of age or less had tumor progression after the first

procedure (60.2%) compared to 24 of 85 children older than 10 (31.2%) (p=0.0004, log-rank). In a Cox model with age as a covariate (not grouped) older age was associated with longer interval to progression (p<0.0001). Location had prognostic significance as well with tumors in the extremities doing better than tumors of the head/neck/face (p=0.0003, log-rank). Extent of resection also had prognostic significance Of 25 cases of complete tumor excision, only 5 progressed (20.0%). Thirty-eight tumors had a near-total resection and 15 (39.5%) of these tumor progressed. By comparison, 74 tumors had a sub-total resection (between 50% and 90%) with 33 (44.6%) progressing. Twenty-one of 31(67.7%) tumors biopsied (less than 50% resection) progressed following the first procedure. These differences are statistically significant with a p<0.0001 (log-rank). Furthermore, for those tumors which progressed, the median time to progression was longer for patients with more extensive resection. Biopsied tumors had a median time to progression of less than 2 years, compared to 5 years for subtotal resection, and greater than 10 years for near total.

Cox models were fit in order to identify possible prognostic factors which predicted the outcome of surgery of plexiform neurofibroma jointly. Age, as a continuous variable, extent of resection, and location were prognostic for shorter interval to progression, even when the variables are considered together. Age was prognostic even in the presence of other variables (p=0.007). In the presence of age, location in the extremities was prognostic for longer interval to progression than other locations; however, the difference between head/neck/face and trunk was no longer significant. In the presence of age, gross total and near total resection were not different from each other in terms of prognosis, but both were different from sub-total resection and from biopsy, which were different from each other. Finally, in a model including jointly age, extent of resection (gross-total and near-total together vs. sub-total vs. biopsy) and location (extremities vs. other locations), age remained significant (p=.003) and gross- and near-total resection had significantly better prognosis than sub-total (p=0.012) or biopsied (p=.001); and tumors in the extremities had significantly better prognosis than all other tumors (p=.05).

DISCUSSION

The conduct of our randomized study of VP-16, IFN, and CRA was adversely affected by three factors. First, we based our estimates of the number of patients with progressive optic pathway tumors on published studies which included clinical criteria for progression as an indication for treatment. By contrast, we required neuroimaging criteria for study entry and it is now apparent that this is a significantly smaller patient group. Second, during the past five years there has developed a growing belief that NF-1 patients with optic tumor have a more indolent clinical course than patients without NF-1. This has engendered a growing reluctance to these tumors in a potentially aggressive fashion. Third, encouraging results of a clinical trial which used carboplatinum and vincristine to treat patients with low-grade gliomas (including optic pathway tumors) was published during the first year of this trial. These findings materially reduced enthusiasm of referring physicians for biological agents such as IFN and CRA which had not established a clinical role in the treatment of glial neoplasms.

Our randomized study in patients with NF-1 and optic pathway tumors does not indicate a significant degree of clinical activity for oral VP-16, IFN, or CRA. However, the small number of patients enrolled in this trial does not permit an accurate estimation of activity. Nor does it allow

us to conclude that these agents are ineffective against optic pathway tumors in NF-1 patients. Rather, we can concluded that study of these agents, either individually or in combination, requires a clear demonstration of their clinical value in non-NF-1 patients before the NF-1 clinical community is willing to proceed with a treatment study of this tumor.

Our randomized study of IFN and CRA in NF-1 patients with plexiform neurofibromas did not identify any patient with objective evidence of tumor volume reduction after treatment. As noted above, the hypothesis to be tested in this clinical trial was that 13-cis-retinoic acid or interferon a-2a would produce objective evidence of tumor response (defined as a decrease in tumor size by 50% or greater) against actively growing plexiform neurofibromas in patients with Neurofibromatosis type 1. As no such activity was demonstrated, our study confirms the null hypothesis. However, results from this study provide potentially important insights into the design and conduct of future NF-1 clinical trials. These are enumerated below:

1. Trial Design. The treatment of malignant solid tumors is a poor model for a trial of PN in NF 1. Unlike cancer, residual tumor in NF 1 is not necessarily fatal, and prolonged tumor control is potentially a positive outcome. A number of the patients on the first study report that treatment has resulted in the longest period of stable disease, and for some the longest interval between surgeries.

2. Defining Endpoints. The number of treated patients who progressed after initiation of treatment was less than expected, judging from retrospective data on tumor progression following surgery in a similar population.

3. Assessment of Response. Tumor measurement was difficult due to the nature of the tumor and may have contributed to a discrepancy between clinical activity and radiographic response. These tumors are irregular in shape and it can be technically difficult to position the patients on the MRI gantry exactly for serial exams. Subtle changes would be difficult to appreciate. Minor responses (25 - 50% tumor shrinkage) which have been considered a response in optic pathway tumor, another slow growing neoplasm common in NF 1, would be difficult to assess in this tumor.

4. Toxicity. Toxicity was manageable, and reversible, but approximately one-third of patients withdrew due to discomfort, on average at the six month interval.

5. Accrual Goals. Interest in the study was high both among physician and patients leading to rapid accrual. Although we proposed to enroll 30 patients in 3 years on the first study, we did so in 10 months. This rapid accrual was achieved despite having only the eastern United States well represented by the study centers.

6. Control Data. There are insufficient data regarding the rate of progression of PN in the untreated state, and little information outside of our retrospective experience at CHOP regarding the prognostic factors that predict progression. In a single arm phase II study, the investigator needs to know what the expected outcome would be if the patient were not to be treated. In the case of patients with recurrent cancer, the expected outcome is tumor growth and death. In patients with plexiform neurofibroma, there are no solid data

for patterns of growth in untreated patients. The only acceptable solution is a design that includes an untreated control group.

These clinical responses and the evidence of tumor stabilization are important in that they suggest that it may be possible to halt growth of PNs with medical therapy. If so, this could have a major impact on patient management. When faced with a patient with a progressive plexiform neurofibroma, who is not a suitable candidate for surgery because of age, location, or the likelihood of radical resection, the treating physician could use medical therapy to delay surgery until the patient is older and more likely to benefit with long term tumor control.

There are insufficient data regarding the rate of progression of PN in the untreated state, and little information outside of our retrospective experience at CHOP regarding the prognostic factors that predict progression. In a single arm phase II study, the investigator needs to know what the expected outcome would be if the patient were not to be treated. In the case of patients with recurrent cancer, the expected outcome is tumor growth and death. In patients with plexiform neurofibroma, there are no solid data for patterns of growth in untreated patients. The data gleaned from the surgical experience at CHOP provide for some comparison, but patient selection for surgery (over a 20 year period) was likely to be subjective and variable, and not necessarily comparable to patients who will enroll on a treatment study. The only acceptable solution is a design that includes an untreated control group. Based on our experience in patients with solid tumors, and our explicit discussions with physicians in our multi-institutional consortium, any randomization of NF-1 patients with progressive PNs to a non-treatment, observation only arm of a clinical trial will be difficult for both patients and physicians to support. By contrast, there is great interest in the clinical community and in NF-1 patients with PNs for new treatment trials. Interest in our study was high both among physician and patients leading to much more rapid accrual than we originally projected. Although we proposed to enroll 30 patients in 3 years on the first study, we did so in 10 months. This rapid accrual was achieved despite having only the eastern United States well represented by the study centers.

The clinical responses and the evidence of tumor stabilization observed in our randomized study of CRA and IFN in NF-1 patients with plexiform neurofibromas are important in that they suggest that it may be possible to halt growth of PNs with medical therapy. If so, this could have a major impact on patient management. Despite this, a number of patients elected to discontinue therapy early. It is clear to us that the factors which motivate a patient with life-threatening cancer to persist with treatment despite some discomforts are significantly greater those for non-life-threatening plexiform neurofibromas and this consideration must be accounted for in subsequent clinical trials.

When faced with a patient with a progressive plexiform neurofibroma, who is not a suitable candidate for surgery because of age, location, or the likelihood of radical resection, the treating physician may elect use medical therapy to delay surgery until the patient is older and more likely to benefit with long term tumor control. In our study, the treatment toxicity was modest, and where present, reversible. However, when all the prognostic factors in our retrospective study are combined, a cohort of NF-1 patients becomes apparent who are unlikely to have long term benefit following surgery; i.e. children less than ten year of age who have lesions of the head,

neck, face, and trunk. Not surprisingly, many will not have a complete resection. For these patients there is a clear need for medical therapy.

Results from our multi-institutional clinical trials in patients with NF-1 suggest that 13-cis-retinoic acid and interferon a-2a may alter the growth patterns of these tumors. In non-neoplastic tumors there are two potential benefits from medical therapy. Obviously any medical therapy which can cause regression would be a tremendous asset to the patient with plexiform neurofibroma. Such a therapy could render the surgically inoperable lesion completely resectable. A more modest goal would be to find an agent which is able to arrest tumor growth. This would allow a delay in therapy for the youngest patients until an age at which tumor recurrence may be less likely. It is not yet known whether arresting growth until beyond age 10 will change the long-term outcome of surgery or whether there is a biologic difference in tumors which present and progress at younger ages. Further efforts in this direction will be required to compliment the surgical approach.

III. NEUROIMAGING STUDIES IN NF-1 OPTIC PATHWAY TUMORS AND BRAINSTEM TUMORS

A. INTRODUCTION

The major goal of our neuroimaging studies, as stated in our original application, was to integrate non-invasive neuro-imaging and laboratory studies of tumor biology with new therapeutic approaches to optic pathway tumors (OPT) and plexiform neurofibromas (PN) thereby providing more accurate predictions of tumor growth and response to treatment, and directly improving the clinical outcome for NF_1 patients. This goal, as originally conceived, could not be achieved because of inadequate numbers of NF_1 patients with progressive tumors (both OPT and PN) during the period of study. However, despite the limited number of patients with tumor progression, we modified our objectives to permit the study of nearly 100 patients with NF_1. Our patient numbers were more than sufficient to allow us to summarize new observations and generate new hypotheses. Accordingly, we were able to make potentially important scientific observations relevant to future NF_1 research from both the neuro-imaging and treatment arm.

This research trial represents the largest pediatric NF1 neuro-imaging study combining innovative neuroimaging techniques including three dimensional (3D) multivoxel proton spectroscopic imaging (^{1}H-MRSI), fluorodeoxyglucose (FDG) positron emission tomography (PET), and dynamic blood flow imaging to begin to characterize NF_1 imaging abnormalities, specifically NF_1 related tumors, in a non-invasive way.

We will began this final report by describing what we have learned and then detail our methods, results, scientific innovations and future directions. The results to be described in this document have relevance to NF_1 in the following critical ways:

(1) Multi- modality imaging of central nervous system pathology in NF_1 pediatric patients can be done in a safe and non-invasive manner.

(2) NF_1-related brain tumors can be characterized with specific biochemical and metabolic profiles and can be differentiated from focal areas of signal intensity (FASI) on T2 weighted MRI.

(3) Implication of the role of the thalamus in NF_1 -related neuropathology and clinically relevant neurocognitive dysfunction.

(4) Demonstration of the importance of longitudinal, cooperative studies to understand the natural history of both central nervous system tumors and plexiform neurofibromas in patients with NF_1.

As a result of this research grant, we were able to generate the following new hypotheses to be tested in future NF1 studies:

1. Metabolite levels in central nervous system tumors of NF_1 specifically low grade astrocytomas of the brainstem and optic pathways, may be substantially different from normal non NF_1 low grade astrocytomas and may help to explain the biochemistry and behavior of NF1 related brain tumors.

2. Specific metabolite profiles identifiable in brain tumors in NF_1 patients may allow for differentiation from surrounding focal areas of increased signal intensity (FASI) seen on T2 weighted MRI.

3. Metabolite levels in the thalamus and other brain regions of NF_1 patients, even those with normal appearing MRIs, may be substantially different than normal non NF_1 controls.

4. Decreased glucose uptake in the thalamus and other brain regions on FDG PET in NF_1 patients may correlate with abnormal metabolite levels of choline, creatine and N-acetylaspartate on 1H-MRSI.

5. Decreased glucose uptake in the thalamus and other brain regions on FDG PET in NF_1 patients may be correlated with FASI on MRI and clinical neurologic and neurocognitive dysfunction.

B. METHODOLOGY

1. Magnetic Resonance Spectroscopic Methods:

Many technical modifications have been introduced into this protocol over the three year period. At the project's inception, Siemens had provided a long echo time CSI sequence and in fact, short echo time sequences were not available. We modified the Siemens CSI sequence to a Te of 40 ms for this study to analyze glutamine and glutamate.

The following details the rationale for our technical modifications and the selection of the short TE. Spectra obtained by CSI measurement can be carried out with different echo times (TE). Spectra obtained by using long TEs (135 ms or 270 ms) contain weaker signal. Choline, Creatine, N-acetyl aspartate, and lactate are metabolites that can be diluted with long TEs and still can be detected with baselines that are flat and well defined. By contrast, glutamine and glutamate have short T2s and cannot be measured with long TEs. Since in vitro data has suggested that the glutamate/glutamine ratio may be an important prognostic indicators in brain tumors, an additional goal of this project was to evaluate glutamate/glutamine levels not well studied in central nervous system (CNS) tumors especially in pediatric patients. As a result, a short TE (<50 ms) CSI was needed to detect signal from glutamine and glutamate because these metabolites have short T_2 relaxation times. The data obtained with the short T_2 s contained more information and the signal to noise ratio (SNR) was better. The disadvantage of using the short T_2s echo times included a baseline effect that was not well-defined with broad signals from proteins. In addition, the lipid signal may become more prominent thereby obscuring both lactate and N-acetyl aspartate. Lipid signals may also appear at longer echo times. In normal brain tissue, the signal from lipids is generally weak, but in brain tumor studies, the lipid signal is often larger containing more NMR visible lipids. Fatty tissue near the tumor may also contribute to the signal and compound the problem. As a consequence, lactate and N-acetyl aspartate levels were not reliably determined.

A spin echo CSI sequence with an echo time (TE) = 40 ms and repetition time (TR) =1600 ms has been used to date. The sequence was obtained by modifying a spin echo CSI pulse sequence provided by Siemens with a long TE (135-270 ms). The region of interest (ROI) was selected by a double spin echo (90∞-180∞-180∞) sequence. The CSI sequence consisted of 16x16 phase encoding steps. Two acquisitions were averaged to accumulate a good signal to noise ratio. The voxel sizes for the measurement were typically 14x14x15 mm^3 or 14x14x12 mm^3. We used a TR = 1.6 sec for this data acquisition and CSI data was collected in 14 minutes but was generally much longer. A reference CSI scan was also collected for eddy current correction and for internal water signal calibration. This reference CSI scan is acquired without water suppression with a small flip angle (10∞-180∞-180∞). Because the flip angle is small, a shorter TR = 0.82 sec was used to go through 256 phase encoding steps in three and a half minutes. The saturation factor of water signal under steady state is only about 1% assuming the water T1 is one second. In addition, we made the assumption that the NMR visible tissue water content is 70%. The water signal amplitude was then averaged over all voxels to calibrate the absolute signal intensity of the metabolites in each voxel. Thirty minutes was generally required for the CSI measurements, including 10 minutes to set up the parameters and for shimming and 20 minutes for data acquisition. All studies were performed on a Siemens Magnetom SP 1.5 T whole body MR scanner at the MRI unit at The Children's Hospital of Philadelphia. The pulse sequence was first tested on a phantom. The change of signal intensity from voxel to voxel on a uniform phantom has a standard deviation of 15%.

The MRS raw data was transferred to a SUN Sparc Station for processing. Data processing software was written in IDL (Interactive Data Language, Research Systems, Boulder, Colorado). MRS results areexpressed as levels of metabolites in each voxel. The numbers have millimolar units. The data acquired in the reference scan was used as an internal reference

[Christiansen *et al*, 1993] for metabolite level calibration. The numbers reported in this study, are lower than the real concentrations because the relaxation effects on the signal intensities are not corrected here. When these effects were corrected, the values agreed with established normal values of metabolite concentrations.

The time domain signal for each voxel was first reconstructed for both CSI spectral data and for the reference scan. The reference signal was used to correct the eddy current effects [Klose, 1990] and to normalize the signal intensity of the spectra (Christiansen *et al*, 1993). The corrected time domain data was then multiplied by a gaussian to enhance signal to noise (width = 300 ms) and Fourier transformed to frequency domain. The phase and baseline of the spectra for each voxel was manually adjusted.

A curve fitting routine was used to calculate the area of myo-inositol (3.55 ppm, 2 protons per molecule), choline containing compounds (3.2 ppm, 9 protons per molecule), creatine and phosphocreatine (3.0 ppm, 3 protons per molecule), glutamine and glutamate (2.0-2.5 ppm, complicated line shapes), N-acetylaspartate (2.0 ppm, 3 protons per molecule). The curve fitting of a short TE spectrum is not a trivial procedure. Each metabolite may have more than one resonance peaks and many metabolites contribute to the spectrum. Two simplifications to analyze CSI data are commonly made by investigators in this field and we adapted these two approaches: First, only contributions from major metabolites were analyzed. Other metabolites including glycine, GABA, and glucose were ignored, because their contribution is small and do not overlap significantly with other peaks. Secondly, we only quantified one component for each molecule. For example, the area of the creatine CH_3 peak at 3.0 ppm is the only peak quantified so that the creatine CH_2 peak at 3.9 ppm was not quantified. The signal from NAA at 2.6 ppm was also not used. As noted above, our objective was to collect the most interpretable data for subsequent statistical analysis.

The spectrum, divided into a three segment curve fitting, was performed on each segment. The frequency range from 1.8 to 2.8 ppm was fitted for glutamine, glutamate and NAA. In the short echo time spectrum, NAA overlapped with glutamine and glutamate. It was therefore necessary to consider all three metabolites together. We assumed that NAA was a single line centered at 2.0-2.05 ppm. The glutamine and glutamate line shapes were measured from 50 mm solutions at a pH = 7.0, using the same MRS pulse sequence. The frequency range from 2.85 to 3.35 ppm contained choline Ch_3 (3.2 ppm) and creatine CH_3 (3.0 ppm). Each metabolite was presented by a single peak and this range is fitted by these two metabolites. The frequency range from 3.35 to 4.0 contains myo-inositol (3.55 ppm). The CH proton of glutamine and glutamate and even glucose together form a broad component at about 3.7 ppm with the CH_2 of creatine at 3.9 ppm The peak areas of myo-inositol were obtained from the curve fit and area of the other two peaks were not used but all overlapping peaks from myo-inositol were considered together.

When a tumor was large enough to occupy several voxels, spectrum with the lowest NAA/Cho ratio were used to represent the tumor. Control values were obtained by using the average of voxels free of tumor and CSF space based on MRI.

As research progressed we implemented three dimensional (3D) proton magnetic resonance spectroscopic imaging (^{1}H-MRSI). Similar to CSI, ^{1}H-MRSI was incorporated into the global MR examination (MRI and perfusion) to take advantage of the fact that the patients were already sedated and in the imager. Combined standard MRI, perfusion MR, and 1H-MRSI in our patients required approximately 75 minutes (35 minutes longer than the standard MRI alone). The MRI, was composed of T_1 weighted sagittal, proton density, T_1 and T_2 weighted axial spin echo, post gadolinium-DTPA injection hemodynamic imaging, and post gadolinium T_1 weighted imaging (approximately 40 minutes). The MRSI which included shimming, selection of the VOI and the actual acquisition currently required another 40 minutes. All studies were performed in the MRI unit of the Children's Hospital of Philadelphia, on the 1.5 T Siemens Magnetom Vision system. A circularly polarized adult head coil was used for both imaging and spectroscopy. Sedation was used for young children with NF1 unable to stay still in the magnet. When necessary, sedation with nembutal was given, it did not exceed the maximum (6 mg/kg). With such sedation, most children slept without difficulty through this 80 minute examination.

The MRI parameters included: field of view = 220 mm, slice thickness = 5 mm, and matrix size = 256*256. For T_1 weighted images, TR=600 ms and TE = 15 ms was used. Proton density and T_2 weighted images was acquired with fast spin echo sequences and TR=3000 ms and TE=20 and 90 ms, respectively. Gadolinium-DTPA was injected after the MRSI examination.

The ^{1}H-MRSI studies were able to simultaneously assess hypometabolic regions identified on FDG PET and focal area of increased signal intensity (FASI) identified on MRI. Average metabolite values from voxels in the thalamus were acquired from both FASI + and FASI - voxels. An FASI + voxel was defined as a region of increased signal on T2 weighted MRI occupying > 50% of the voxel. An FASI - voxel was defined as no increased signal on T2 weighted MRI in the voxel. ^{1}H-MRSI metabolite peak areas were described in arbitrary units and ratios for both FASI + and FASI - voxels in the thalamus. 2D CSI which acquired spectra from an array of voxels but is limited to one plane and NF1 patients may have imaging (MRI or PET) abnormalities in more than one location, hence we took advantage of the three dimensional technique. Our results indicate that "State of the art" ^{1}H-MRSI best meets the requirements of NF1 abnormalities demonstrated at multiple levels.

1H-MRSI allows coverage of a three dimensional volume of interest (3D VOI) with multiple single slices sequentially interleaved. One disadvantage of slice-interleaving is it is inefficient in signal-to-noise-ratio (SNR) per unit-time. As a consequence, to obtain a reasonable voxel SNR, a time requirement of approximately 40 minutes in addition to other time constraints (time for patient loading, coil tuning, imaging and shimming) brings the total examination time to at least 100 minutes. This poses a considerable obstacle in children. The children are lightly sedated and testing is aborted when the sedation wears off. Under these constraints, the MRSI examination must be made as brief as possible for patient comfort while simultaneously preserving the scientific information acquired. To address this technical problem, hybrid of 2D-CSI with 1D HSI to achieve simultaneous 3D coverage of the VOI was accomplished by Drs. Z Wang and O. Gonen. 3D coverage has the advantage of providing the same voxel SNR as the "current-art" N=4 multislice-interleaved acquisition of similar resolution in a quarter of the time, making this procedure particularly well suited for pediatric settings in general.

The 3D ^{1}H-MRSI measurement was performed with a hybrid shown in the Appendix. A 135 ms echo time was used. The general form of the MRSI localization sequence, was retained throughout for data-computability reasons as well. The 135 ms echo time was selected for higher measurement precision for two reasons: 1) better definition of baseline; and 2) less interference from other peaks. A test on a uniform phantom has demonstrated that detection sensitivity for different voxels are uniform on our scanner, with the standard deviation less than 1.5% within one slice, excluding voxels at the edge of the PRESS volume in the XY plane. As a result, signals from various voxels can be directly compared with each other. The ^{1}H-MRSI parameters was 16x16 phase encoding steps with a field of view of 16 cm and slice thickness of 15 mm, translating into a voxel size of 1x1x1.5 cm.

The position of the patient did change through the entire MRI/MRSI session. The MRSI study includes approximately 5 to 10 minutes for setting up positions and shimming followed by about 27 minutes for collecting the spectra. The selection of volume of interest is image-guided by a neuroradiologist investigator.

Two normalization factors were taken into account in order to compare signal intensity for different patients (intersubject variability) and for the same patient over time (intrasubject variability). First, the RF coil loading was accounted for by multiplying the signal by the RF voltage needed for a 90° pulse of fixed length (inversely proportional to the detection sensitivity). Secondly, the possible instability of the MRI scanner was accounted for by a bi-weekly calibration.

2. MR Perfusion Methods

The particular MRI approach utilized here required a bolus of contrast agent specifically gadolinium-DTPA (Magnevist) injected into a vein. The initial 'first pass" passage of this indicator through the brain is studied by taking a succession of images in the brain at the rate of approximately one image every second. The effect of the gadolinium is to shorten both the Tl and the T2* relaxation times of the tissue. Conventionally, the passage of gadolinium is studied using the T2* effect (Edelman *et al,* 1990). The concentration (C) of gadolinium at any time (t) following injection is proportional to the change in the relaxivity of the tissue (DR2*) in the range used clinically (Villringer *et al*, 1988). DR2* can be measured from the intensity of the signal before any gadolinium is injected (So), and the signal at time t (St).

C is proportional DR2*. $DR2^* = \ln(So/St)/TE$

The mathematics of following the MR contrast is the same as that worked out by Axel for CT studies of flow using x-ray contrast media (Axel, 1980). The flow (ml blood/ml tissue/sec) can be calculated from the time course of the contrast agent in the tissue and the arterial input time course (Perman *et al*, 1992). This calculation assumes that the bolus is instantaneous yet in clinical practice, the injection is not instantaneous. A more convenient and feasible measurement in children that we have employed was to determine the relative blood volume in the tissue (RBV) from the DR2* - time curve following the bolus injection. The overriding advantages of using this indicator approach with gadolinium are: one, this approach is

easy to apply clinically: two, it requires no extra patient time in the scanner because the gadolinium is injected as part of the clinical study; and three, the signal change (often about 30%) is significantly larger than the produced by the techniques that label the blood using r.f. saturation (usually 1-2 %). One disadvantage to the gadolinium bolus approach is that it is not appropriate for functional studies of multiple tasks but is useful for a single study of resting flow to tissue such as we propose in this application. The second disadvantage is that the equations assume that the contrast agents flow through the brain only once yet recirculation of the blood does occur and increases the concentration measured during the tail of the time-course curve. Some groups have answered this problem by fitting the rising part of the curve (assumed to be uniquely arising from the first pass) to a theoretical 'gamma' curve. While there are disadvantages to the use of the gadolinium bolus approach to flow measurements, (Belliveau *et al*, 1990; Weisskoff *et al*, 1993) these problems are not sufficient to prevent its usefulness in our patient population.

Patients were imaged using the same rapid gadolinium bolus described in the Preliminary Studies. Echo planar images (EPI) will be acquired on a Siemens 1.5 Tesla Vision System and transferred to a SUN workstation for post analysis. It is necessary (in principle) to integrate the whole of the area of the excursion of the image data from the baseline but again errors can be introduced by the tail of the curve. For this reason, several groups have made use of a gamma fitting algorithm that fits the rising portion of the curve to a theoretical curve, while other groups have suggested that it is sufficient to measure either the maximum excursion or the maximum rise rate of the signal in question. During the period of this grant, we investigated which of these various approaches gave the best relative measurements of the gray and white matter, and then applied the technique to measuring the RBV of the thalamus, gray and white matter for these patients.

In addition, comparison of regional metabolite measurements obtained using PET and MR perfusion imaging were carried out using ROI analysis. This approach avoided registration errors which may have been encountered while attempting to compare perfusion images obtained by differing modalities on a pixel by pixel basis, while providing a functionally relevant basis for comparison.

3. FDG PET Methods

The FDG method to determine regional cerebral metabolic rates for glucose was introduced by investigators at PENN in 1976 and has been utilized extensively and validated at the University of Pennsylvania. This validation has been carried out in both normal resting and activation studies as well as in disease states. Absolute quantitative studies require insertion of an arterial line, which is invasive and in our experience, is neither feasible nor warranted in children. In addition, absolute metabolic rates appear to vary considerably among and within subjects in both normal and patient populations.

We used both qualitative (visual interpretation) as well as quantitative approaches (described above) to determine the metabolic activity of the regions of interest. Qualitative assessment will use the following grading system: 1 = totally absent uptake, 2 = slightly less uptake than surrounding area, 3 = same uptake as surrounding area, 4 = slightly to increased

uptake compared with surrounding area, and 5 = markedly increased uptake . Quantitative assessment will include measurements of FDG counts and calculated ratios of FDG counts in the regions of interest to whole brain. In addition to the tumor regions, there are 90 regions of interest but for statistical analysis the following regions have been analyzed:

- Frontal gray matter/white matter
- Parietal gray matter/ white matter
- Temporal gray matter/white matter
- Occipital gray matter/white matter
- Caudate nucleus
- Putamen
- Globus Pallidus
- Thalamus
- Corpus Callosum

A single venous catheter was inserted into an antecubital vein of one arm for the administration of FDG. No arterial line to withdraw blood samples was utilized for this research. A second venous line was used initially in the first 17 studies. All patients who required sedation were sedated with pentobarbital at identical doses to those used in MRI scan sedation. The sedation was initiated at least 40 minutes after the administration of FDG and before the imaging was started. FDG was administered as a bolus 30 uci/kg (25% of the standard dose) because of the high sensitivity of the HEAD-PENN-PET scanner. Forty minutes after the administration of FDG, the patient was positioned into the HEAD-PENN-PET scanner. The PET scans were acquired parallel to the canthomeatal line and included the entire brain and the upper cervical spinal cord (the axial field of view for this instrument = 26 cm). The total imaging time was 30 minutes which in our experience was tolerated well by our pediatric NF1 patients.

D. Results

1. Patient Characteristics / Patient Accrual

A total of 35 NF_1 patients with central nervous system tumors have been enrolled on either the treatment or neuro-imaging arm of this study (Appendix #).

Twenty-five NF_1 patients filled 29 neuroimaging positions. Ten patients had brainstem tumors. Nineteen patients had optic pathway tumors. Twelve patients had newly diagnosed optic pathway tumors and seven patients had progressive optic pathway tumors. Since four of 12 patients entered originally as newly diagnosed optic pathway tumors eventually progressed, they were re-enrolled on the progressive optic pathway tumor arm. As a result, 25 NF_1 patients were enrolled in 29 neuro-imaging slots. Patient characteristics are detailed in (Appendix #). In addition, eight patients had both brainstem tumors and optic pathway tumors.

There were 15 males and 10 females. Twenty-four patients were white, not of Hispanic origin and one patient was black, not of Hispanic origin.

All patients met the clinical criteria for Neurofibromatosis Type1, established by the National Institutes of Health Consensus Development Conference (1987), by virtue of at least two of the following findings: multiple cafe-au-lait spots (six or more >0.5 cm if postpubertal); two or more subcutaneous neurofibromas or one plexiform neurofibroma; axillary or inguinal freckling; optic gliomas; tibial pseudarthrosis or sphenoid wing dysplasia; two or more Lisch nodules; and a first-degree relative with the disorder.

Brainstem tumors were defined as diffuse or focal mass lesions of the midbrain, pons, and medulla. To distinguish brainstem tumors from the typical NF_1 high-intensity foci on T_2-weighted MRI (i.e., UBOs), we used established neuroimaging criteria to describe these foci; (1) frequent isointense appearance on T_1-weighted MRI; (2) multiple, bilateral intracerebral distribution; (3) absence of mass effect; and (4) absence of gadolinium enhancement. For all brainstem and optic pathway tumors, the MRI region of interest (ROI) selected, was greater than 1 cm in 24 of 25 (96%) patients, with missing data on one patient. Twenty three of twenty four patients (92%) had decreased T_1 signal in the ROI and 23 of 24 patients had increased T_2 signal in the ROI on MRI imaging. Seventeen of 24 patients (68%) had gadolinium enhancement in the ROI. Seven patients had ROIs that did not enhance with gadolinium.

2. Magnetic Resonance Spectroscopy Results

A total of 25 NF_1 patients have been studied with proton magnetic resonance spectroscopy, either with single slice CSI, multi-slice 1H-MRSI, or single voxel MRS. Fifty one imaging studies have been attempted (**Appendix #**). Ten were inadequate due to technical difficulties (**Appendix #**). Seven additional studies analyzed with three dimensional (3D) proton magnetic resonance spectroscopic imaging (1H-MRSI), our newest technical modification are also reported **(Appendix #).**

A total of 19 NF1patients were studied with short TE chemical shift imaging on 44 exams **(Appendix #).** The tumors studied were divided into two categories according to tumor location in the optic pathway or brainstem **(Table # 1).** While eight patients had tumors in both locations, the single slice CSI pulse sequence can only measure tumor at one location in one study session, hence only one tumor was studied.

Interpretable data was obtained from 34 CSI examinations including 21 studies of 13 optic pathway tumor patients and 13 studies of 6 patients with brainstem tumors **(Table # 1).** Of the 44 studies analyzed to date, CSI could not be achieved in ten patients. Four patients had dental braces or other metal implants near the MRS region of interest and shimming was difficult. In two of these patients, we used single voxel techniques when shimming for CSI could not be achieved. The studies with single voxel technique will not be reported here. In five studies, patient motion during the exam due to inadequate sedation, resulted in discontinuation of the study, or unreliable data. In one study, the data was lost because of data corruption in the primary optical storage disk prior to data transfer.

Table #1. Tumors Analyzed with CSI Technique (Single Slice/ Multi-Voxel)

Tumor Location	Number of Patients	Number of Studies
Optic Pathway	13	21
Brainstem	6	13
Total	19	44

Metabolites were measured for all voxels in a single slice. Voxels were characterized as: (1) no tumor present or $\leq 25\%$ filling the voxel; (2) tumor filling voxel $\geq 25\%$ or $\leq 50\%$; (3) tumor filling voxel $\geq 50\%$ or $\leq 75\%$; (4) tumor filling voxel $\geq 75\%$ or $\leq 100\%$; or (5) tumor completely filling voxel. For this report, only voxels completely filled with tumors were analyzed.

Table 2a MRS Study #1

Number of Voxels with 100% Tumor Present					
	0	**1**	**2**	**3**	**4**
301	-	1	-	-	-
302	1	-	-	-	-
303	1	-	-	-	-
304	-	1	-	-	-
305	1	-	-	-	-
306	-	1	-	-	-
307	1	-	-	-	-
308	1	-	-	-	-
401	-	-	1	-	-
402	-	-	-	1	-
403	-	1	-	-	-
404	-	-	1	-	-
405	-	1	-	-	-
406	1	-	-	-	-
407	1	-	-	-	-
408	1	-	-	-	-
409	1	-	-	-	-
410	1	-	-	-	-
501	-	1	-	-	-
502	-	-	-	-	-
503	-	-	1	-	-
504	-	-	-	1	-
505	-	1	-	-	-
506	-	-	-	-	1
507	1	-	-	-	-
Total	11	7	8	3	4
Twenty-five patients studied. Eleven patients had tumor < 100% of the voxel. Fourteen patients had 22 voxels with tumor completely filling the voxel.					

Table 2b MRS Study #2

Number of Voxels with 100% Tumor Present			
	0	**1**	**2**
301	-	1	-
302	1	-	-
303	1	-	-
304	1	-	-
305	1	-	-
306	1	-	-
307	1	-	-
308	1	-	-
401	-	1	-
402	-	-	1
403	-	1	-
404	-	-	1
405	1	-	-
406	-	1	-
407	1	-	-
408	1	-	-
409	1	-	-
410	1	-	-
501	1	-	-
502	1	-	-
503	1	-	-
504	1	-	-
505	1	-	-
506	1	-	-
507	1	-	-
Total	19	8	4
Twenty-four patients studied. Nineteen patients had tumor < 100% of the voxel. Six patients had 12 voxels with tumor completely filling the voxel.			

Table #3 Metabolites in all patients with either brainstem tumors or optic pathway tumors (only voxels with tumor filling voxel were analyzed)

	First MRS Study			Second MRS Study		
	Mean	**SE Mean**	**S.D**	**Mean**	**SE Mean**	**SD**
Myoinositol	*3.16*	0.47	1.62	*2.56*	0.61	1.50
Choline	*1.76*	0.19	0.67	*2.01*	0.18	0.43
Creatine	*3.95*	0.55	1.89	*3.82*	0.62	1.53
Glutamate	*1.34*	0.39	1.37	*1.91*	1.02	2.49
Glutamine	*4.66*	0.98	3.47	*2.34*	0.63	1.53
N Acetyl Aspartate	*3.40*	0.58	1.94	*3.83*	1.11	2.73

* 24 patients * 25 patients

Brainstem tumor results utilizing CSI technique:

The first group of NF1 patients with CSI examination had tumor in the brainstem, often with extension to the cerebellum (Table #4). Our accrual goal of ten NF1 patients with brainstem tumors was achieved. We summarize the results from our patients in Table # 2. For these patients, choline was significantly higher in the tumor than control ($p<0.03$). When tumor regions were compared with control regions, creatine and NAA were both significantly decreased with p values 0.01 and 0.02 respectively. Ratios of Cr/Cho and NAA/Cho were also decreased significantly with p values <0.001 for both ratios. While there were insufficient patient numbers in the brainstem tumor group to detect a difference between progressive and non-progressive disease across the group, significant differences between tumor and control regions in single subjects were noted.

Table # 4
Brainstem Tumors Compared to Control Spectra Analyzed by CSI Technique (Intra-subject Evaluation).

Metabolite	choline	creatine	NAA	Cr/Cho	NAA/Cho
Tumor	2.2±0.5	4.1±1.3	3.5±2.2	1.8±0.6	1.6±1.0
Control	1.8±0.6	5.3±1.1	5.2±1.9	3.1±0.9	3.0±1.1
p-value	0.03	0.01	0.02	0.0004	0.001

Data is based on the patients with 13 CSI exams of their brainstem tumors.

Optic pathway tumor results utilizing CSI technique:

Twelve patients with newly diagnosed optic pathway tumors and seven patients with progressive optic pathway tumors were accrued for a total of nineteen NF1 patients. Four patients were on both arms because they began as newly diagnosed optic pathway tumor patients and then had progressive disease. We report our data based on the metabolites in 20

studies in 12 patients with optic pathway tumors (both newly diagnosed and progressive optic pathway tumor). Only one study has inadequate data.

In NF1 patients with chiasmal tumors, the tumor was usually smaller than the size of one voxel. The remaining space in the voxel was occupied by CSF partial volume (typically 15-25%), thus all metabolites may appear to have a lower intensity. We divided all metabolite levels in chiasmal tumor by 0.8 to correct for this effect. Tumor in the optic tracts or optic radiations were often large enough to fill a whole voxel. Compared with the control spectra, the tumors had statistically significant increase in choline, decrease in NAA and decrease in NAA/Cho ratio (Table # 5).

Table #5.
Optic Pathway Tumors Compared to Control Spectra Analyzed with CSI Technique (Intra-subject Evaluation)

Metabolite	Choline	Creatine	NAA	Cr/Cho	NAA/Cho
Tumor	1.7±0.6	3.7±2.0	3.1±0.6	2.5±1.3	2.1±1.1
Control	1.4±0.4	4.0±1.2	5.0±1.2	2.9±0.8	3.5±1.2
p-value	0.05	0.31(n.s.)	0.0002	0.13(n.s.)	0.0002

Data was based on metabolites from 20 studies in 12 OPT patients. (Missing data on one patient.)

A major objective of this study was to determine whether MRS parameters were correlated with tumor growth and progression across the groups. The average values and standard deviations were calculated for three groups: (1) new tumor diagnosis at study onset; (2) progressive tumor at study onset; and, (3) tumor progression during the study period (Table # 6). The average choline level was the highest for tumors that progressed on study and the lowest for new tumors with no progression. However, no significant differences in average values of metabolite levels or ratios were found between progressive tumors and non-progressive tumors in mean values by ANOVA ($p > 0.05$) for all variables most likely due to the small numbers or limited power of the study. In one patient with progressive disease during the study period and one patient with progression at study onset had metabolite levels generally lower than control brain tissue. Both patients had chiasmal tumor with extension to the optic radiations. In both cases, the optic radiation tumor was measured. One patient with surgical resection for clinical progression had pathologically proven fibrillary astrocytoma.

Table #6
Optic Pathway Tumors Analyzed with CSI Technique (Inter-subject Evaluation)

Metabolites	# of patients	# of studies	choline	creatine	NAA	Cr/Cho	NAA/Cho
New Tumor Diagnosis	4	9	1.5±0.3	3.5±2.0	3.1±1.4	2.6±1.3	2.4±1.0
Tumor Progression at Study Onset	4	6	1.8±0.8	3.7±2.5	2.6±1.7	2.3±1.3	1.8±1.0
Tumor Progression on Study	3	4	2.0±0.8	4.1±1.7	4.3±1.4	2.6±1.4	2.1±1.5
p-values			0.26	0.89	0.31	0.87	0.60

Results utilizing 1H-MRSI technique:

As described in our methodology section as our research progressed, we implemented 1H-MRSI to better characterize those NF1 patients with multiple glial CNS tumors located in both optic pathways and in the brainstem, as well as focal areas of signal intensity (FASI) or unidentified bright objects (UBO) in multiple brain regions.

Seven children with NF1 have been studied utilizing 1H-MRSI to date with tumors of the visual pathways or brainstem. There were inadequate numbers for statistical analysis. Examples of this analysis is evident in three patients as demonstrated in Table #7. In addition, all patients had focal areas of signal intensity (FASI) identified on MR imaging of the thalamus and other brain regions (described below).

Table #7. Tumor Analyzed with 3d Spectroscopic Imaging.

	Patient #	choline	creatine	NAA	Cr/Cho	NAA/Cho
Chiasmal tumor	506	73	116	135	1.69	1.83
	307	49	137	209	2.76	4.23
	303	115	149	120	1.29	1.05
Control regions	506	116±63	238±117	196±106	2.22±0.72	0.79±0.58
	307	57±23	165±55	243±69	3.12±1.08	4.80±2.04
	303	73±22	144±44	222±46	2.1±0.72	3.3±1.1
Additional tumor regions	506	163±63	257±180	165±91	1.62±0.81	1.20±1.02
	303	108±29	194±53	206±38	0.63±0.18	0.69±0.27

* Metabolite levels are listed in arbitrary units.

We have included an image that utilizes 1H-MRSI techniques to illustrate the difficulty in clearly discerning tumor infiltration and normal brain regions from FASI + regions (Fig 1).

Fig.1. Sagittal image showing the placement of the VOI with the four HSI slices. The VOI, tumor (circled) and the FASI (highlighted) are demonstrated (axial T2 weighted FLAIR image / slice #4). The spectra from slice #4 is displayed to the right. The tumor region is marked with an asterisk. The shaded area highlights regions of elevated Cho/NAA in the temporal lobe. This region was specifically selected because it has been read as both "FASI" and "tumor" by two neuroradiologists highlighting the heterogeneity of this disease.

Data utilizing the 1H-MRSI technical modifications in these patients (Table #8) and in two healthy adult volunteers (Table #9) are described in this report. Average metabolite values from voxels in the thalamus were acquired from both FASI + and FASI - voxels. An FASI + voxel was defined as a region of increased signal on T2 weighted MRI occupying > 50% of the voxel. An FASI - voxel was defined as no increased signal on T2 weighted MRI in the voxel. 1H-MRSI metabolite peak areas were described in arbitrary units and ratios for both FASI + and FASI - voxels in the thalamus. The 1H-MRSI data had been normalized by RF loading of the coil.

Our results (Table #8) utilizing 1H-MRSI technical modifications include the following:

1) FASI + voxels in the thalamus had higher Cho and higher Cr compared to FASI - voxels;
2) FASI + voxels in the thalamus had increased Cho/NAA ratios and increased Cr/NAA ratios when compared to FASI - voxels;
3) FASI + voxels in the thalamus had relatively normal NAA similar to FASI - voxels;
4) Even FASI - voxels in the thalamus had increased Cho which suggested a diffuse pathologic process in this region in NF1 patients.

Estimate of Regional Cerebral Blood Flow (RBV) in Three NF1 Subjects

Figure 1a

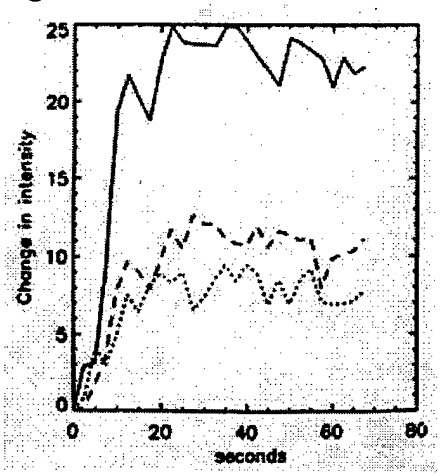


Figure 1b

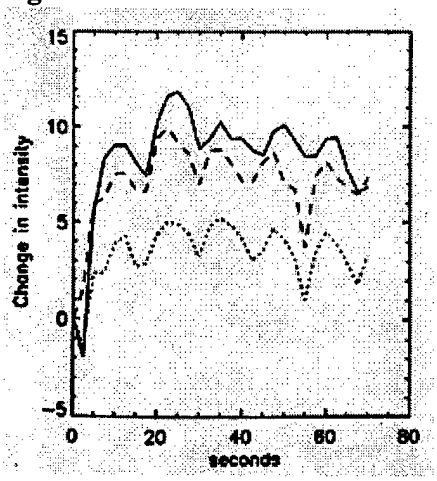
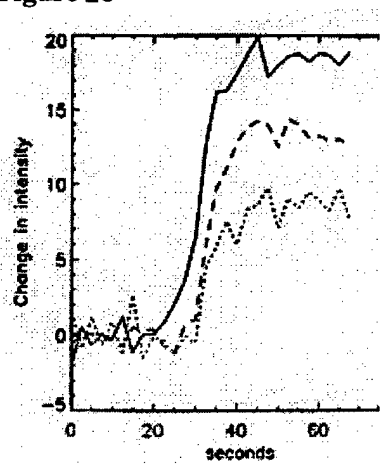
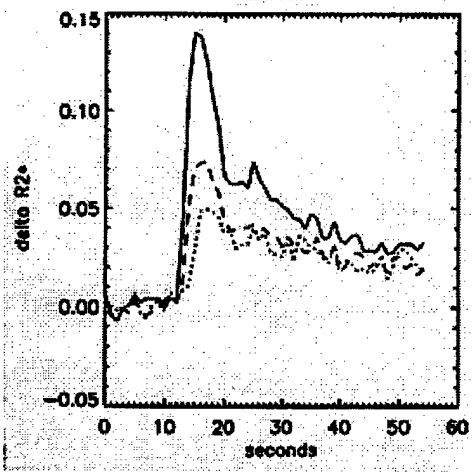


Figure 1c



—— = RBV (grey matter)
- - - - - = RBV (white matter)
..... = RBV (thalamus)

Figure 1.d. Estimate of Regional Cerebral Blood Flow (RBV) in NF1 Subject with Rapid Gadolinium Bolus (< 3 seconds)



—— = RBV (grey matter)
- - - - - = RBV (white matter)
..... = RBV (thalamus)

NF1 Subjects

FDG PET Results

Table #8

Comparison of metabolite levels measured by ^{1}H-MRSI in FASI+and FASI- voxels in the thalamus of five NF1 patients (values are peak areas in arbitrary units).

Army subject #	Subject age	FASI+ voxels (average)					FASI- voxels (average)				
		Cho	Cr	NAA	Cho/ NAA	Cr/ NAA	Cho	Cr	NAA	Cho/ NAA	Cr/ NAA
506	3 years	382	255	318	1.2	0.8	288	190	288	1	0.66
307	3 years	569	292	157	3.62	1.86					
303	3 years	259	172	166	1.56	1.04	249	230	242	1.03	0.95
409	4 years						200	166	206	0.97	0.81
407	10 years	243	193	194	1.25	0.99	205	166	246	0.83	0.67
Average Value		363	228	209	1.91	1.17	236	188	246	0.96	0.77

The normal development of the thalamus as measured by 1H-MRSI has not been reported. It is expected that healthy adults have lower Cho and higher NAA than healthy children. Examples of normal adult metabolite levels measured by 1H-MRSI are provided in table 2b for comparison. Age-matched control studies to validate metabolite data acquired in NF1 patients are needed.

Table #9:

Metabolite levels measured by ^{1}H-MRSI in the thalamus in two adult control volunteers (values are peak areas in arbitrary units).

	Cho	Cr	NAA	Cho/NAA	Cr/NAA
Adult 1, 25 y	129	119	302	0.43	0.39
Adult 2, 35 y	149	122	259	0.58	0.47

Our ^{1}H-MRSI data has demonstrated a pattern of increased choline and creatine in thalamic FASI in NF1 patients analyzed with this technique. We particularly found this pattern of increased choline intriguing, in light of the fact that FASI identified on MRI are postulated to represent demyelination (Sevick, 1992) or abnormal myelin development from delayed glial differentiation (Itoh, 1994). It is documented that disorders of abnormal myelin formation, such as certain leukodystrophies may demonstrate decreased choline (Wang, 1996). In adrenaleukodystrophy, the ratio of choline/creatine is increased in the acute phase, but whether this represents an increase in choline or a decrease in creatine is unclear. In multiple sclerosis, the ratio of choline/creatine is increased presumably, due to a decrease of creatine not an increase in choline (Davies, 1995). Increased creatine as found in FASI identified regions in our studies has not been reported for disorders of myelin to our knowledge. It appears that the ^{1}H-MRSI pattern of increased choline and creatine in FASI is not consistent with the typical spectrum

obtained for either demyelinating or dysmyelinating disorders but again the underlying pathology of FASIs has not been defined.

Implementation of ^{1}H-MRSI in pediatric NF1 patients was important for several reasons: 1) it allowed for the quantitation of metabolites both with absolute signal intensities and metabolite ratios which allowed for improved data analysis when compared to the previous methods of analyzing only simple metabolite ratios; and 2) it provided a noninvasive method of investigating of imaging abnormalities noted on both FDG PET and MRI scans.

3. MR Perfusion Results

Experiments were conducted using the gadolinium bolus technique for measuring perfusion in NF1 children with brain tumors resulting in considerable expertise with this technique. Technical refinements in our perfusion imaging and flow visualization have improved the quantitative characterization of blood flow in children over time.

We will first describe our early efforts. All studies were initially performed utilizing the T1 effect using a T1 weighted inversion recovery turbo-gradient echo sequence (Schwarzbauer et al, 1993). The effective TI = 850ms, the single slice thickness = 5 mm: and the field of view (FOV) was approximately 200-250mm (depending on the size of the child) with a matrix size of 128*128. One image was obtained every 2.5 seconds. Unfortunately, these studies were compromised somewhat by the requirement, that previously existed in our hospital, that pediatric patients were not permitted to have rapid contrast injections. The gadolinium 'bolus' was therefore injected over a period of at least 20 seconds. A total of twenty-five NF1 patients with brainstem and optic pathway tumors have been studied. We found that our blood flow data was flawed by multiple changes in technique so that our results in this part of the grant were less important than the technical refinements we described previously in the interim report.

Again we will demonstrate data from three NF_1 subjects described in the interim report. Figure 2 shows the initial time course of the signal in three NF1 studies. We are still reluctant to derive any quantitative results from our data because of the long time course over which the gadolinium was injected. Nonetheless it is clear that in all patients, the signal changes in the white matter are about half that of the grey matter. When compared to both white and grey matter signal, the signal from the thalamus varies substantially from patient to patient. In figure 2 image a, the signal from the thalamus is similar to that of the white matter, while in image b, the signal appears close to that of gray matter. Finally in figure 2 image c, the signal change in the thalamus is intermediate between gray and white matter. While it is difficult to interpret these data in terms of regional cerebral blood flow/blood volume, it is apparent that evaluation of regional blood flow to the thalamus may be decreased in some NF1 patients.

We have recently developed a hospital approved protocol for injecting the gadolinium rapidly (total time = 3 seconds). Figure 2 d shows the results of measuring the blood flow in an image slice that includes the region of the thalamus in a 10 year old girl with NF1. The patient was positioned in Siemens 1.5 Tesla vision whole body NMR system in a head coil. Sagittal Tl weighted images were obtained for localization of the plane of the subsequent study. 60 T2* weighted echo-planar images were taken in succession with the following parameters: effective

TE=64ms, 1 second between images, FOV=250mm, matrix size 128*128, slice thickness 7 mm. After 10 images had been taken, O.1mm/kg of gadolinium (Magnevist, Berlex) was injected into a brachial vein over a period of 3 seconds. The images, were transferred to a Sun Sparc II workstation via a local hospital network and there analyzed using the programs written in IDL (Research Systems Inc. Colorado). The first five images of the set were ignored because this is the period during which the system is achieving a steady state. Then three regions of interest (white, gray and thalamus) were identified, and the average signal for each area calculated from each image. The set of 55 intensity values were analyzed as a 55 second long time study. The first 5 points were averaged to generate the baseline value (So), and for each time point (t) the change in relaxivity calculated as DR2* = 1n(So/St)/0.064 where St is the intensity of the pixel at time t. Figure 2 d shows the time course of DR2*. The relative flow for each region of interest was then calculated as the sum of the values of DR2* for all 55 time points. From this data we measure that the relative blood volume for gray, white matter and the thalamus are 1.92, 1.05, 1.28. The ratio of perfusion between gray matter and white matter is approximately 2:1 in general agreement with the literature (Wood, 1987).

By the time the new protocol was approved and new techniques implemented so late in the study, our small patient number made further analysis not feasible. The usefulness of the dynamic blood flow technique in the NF_1 patient population to assess areas of hypometabolism noted on FDG PET and FASI demonstrated on MRI remains to be seen but preliminarily looks exciting.

Results from Positron Emission Tomography Studies

The NF_1 research team at The Children's Hospital of Philadelphia (CHOP) was funded by the U.S. Army Research and Development Command to explore the role of modern imaging techniques in NF1 patients with brain tumors. Twenty-five NF1 subjects have completed 35 FDG PET studies. Twenty five patients were enrolled and four patients with newly diagnosed optic pathway tumors who progressed were re-enrolled on the progressive neuro-imaging arm of the trial. Therefore a total of twenty-nine neuro-imaging positions were filled including: ten patients with brainstem tumors, twelve patients with newly diagnosed optic pathway tumors and seven patients with progressive optic pathway tumors.

Of the ten patients with brainstem tumors (Army # 401-410), only two patients had progressive disease. One patient underwent a subtotal surgical resection of a cervico-medullary fibrillary astrocytoma but unfortunately this patient's family refused a second FDG PET study at the time of tumor progression. The second patient with progressive disease and consecutive FDG PET scans had disease in the thalamus not the brainstem, hence it will not be analyzed here. Decreased FDG uptake was noted both by visual grade and by FDG counts in all patients with brainstem tumors and was statistically significant (Table 10a).

Decreased FDG uptake was noted in the brainstem of NF_1 patients whether or not they had brainstem tumors. In the first FDG PET studies in 24 NF_1 patients analyzed, the mean visual grade of the brainstem with or without a brainstem tumor = 2.00 [(SD = 0.67) $p<0.012$] (Table 10a). In addition, there was good correlation between FDG uptake and counts in the brainstem of NF1 patients with or without a brainstem tumor (Table 11). Ten patients had a

second FDG PET study with a mean visual grade in the brainstem of 1.87 (SD = 0.72) (Table 10b).

Of twelve patients with newly diagnosed optic pathway tumors (Army # 301-312), one patient (Army # 306) refused FDG PET study but completed the other neuro-imaging exams. Of twelve patients with newly diagnosed optic pathway tumors, one patient had disease of the thalamus, midbrain, and hypothalamus (Army # 310/ 505). Three patients (Army # 309, 310 & 311) had progressive disease both clinically (visual or neurologic deterioration) and radiographically (increased tumor size greater than 10% on MRI). The first two patients with progressive optic pathway tumors had very metabolically active tumors (Army # 309 & 310). Both patients (Army # 309/310) had biopsy proven fibrillary astrocytomas and one of these patient is in supportive/hospice care (Army # 310). The third patient (Army # 311) with a progressive optic pathway tumor had a PET imaging pattern consistent with a metabolically inactive tumor. One patient with a metabolically active tumor (optic radiations) by FDG PET scanning had no radiographic (MRI) tumor progression but developed seizures although not a clear clinical progression (Army # 301). The remaining 7 patients had optic pathway tumors seen as metabolically inactive.

Of 14 patients with progressive optic pathway tumors treated with 13 cis-retinoic acid, alpha interferon 2A, or etoposide, seven patients also participated on the neuro-imaging arm (Army # 501-507). Four of the 14 patients treated for their progressive optic pathway tumors had disease progression (Army # 105, 108, 109, 113)). The first patient previously on the newly diagnosed optic pathway tumor arm had progressive disease and a metabolically active tumor on FDG PET and the second patient refused further imaging at progression. The remaining five patients had metabolically inactive tumors.

In addition, we report a consistent pattern of decreased glucose uptake in the thalamus in our NF1 patients, which was based on both qualitative analysis (visual grade) and quantitative analysis (ratios of FDG count to whole brain) (see methods). Twenty-five NF 1 patients had 35 FDG PET scans. To recapitulate, all brain regions and tumors were assigned a visual grade (VG) based on FDG uptake (1 = absent, 2 = decreased, 3= normal, 4 = moderately increased, 5 = markedly increased). FDG counts and visual grades were recorded and correlated from multiple brain regions, including: frontal (FR), parietal(PA), temporal (TE), and occipital (OC) lobes, visual cortices (VC), caudate (CD), globus pallidus/putamen (GP) and thalamus (TH) (Table # 10a and b). In addition, counts from the thalamus were compared to counts of the basal ganglia and hemispheres. Comparisons of these averaged counts between thalamus and basal ganglia, and between thalamus and hemispheres were made by means of t-tests. Both paired t-tests and independent t-tests for groups with unequal variances were utilized *(Table #7).* The thalami, visual cortices and temporal lobes had significant hypometabolism that was reflected on consecutive studies. There was also excellent correlation between visual grades and FDG counts based on region of interest analysis normalized to whole brain activity. Independent t-test for groups with unequal variances demonstrated both statistically significant differences between averaged counts of the thalamus and those of the basal ganglia, and also between counts from thalamus and counts from the hemispheres.

Table #10a: Mean Visual Grade on FDG PET (FIRST STUDY)

Location	Mean VG first scan	SD	S.E. Mean	2 - Tailed Significance
Frontal	2.90	0.29	0.06	0.025
Parietal	2.74	0.41	0.08	0.016
Temporal	2.27	0.50	0.10	0.00
Occipital	2.70	0.44	0.09	0.012
Visual Cortex	2.25	0.54	0.11	0.007
Caudate	2.83	0.43	0.09	0.005
Putamen / Globus Pallidus	2.89	0.42	0.09	0.607
Thalamus	1.78	0.66	0.14	0.004
Brainstem	2.00	0.67	0.14	0.012

24 patients first study

Table 10b: Mean Visual Grade on FDG PET (SECOND STUDY)

Location	Mean VG second scan	SD	S.E. Mean
Frontal	2.88	0.23	0.09
Parietal	2.74	0.42	0.13
Temporal	2.22	0.31	0.10
Occipital	2.63	0.52	0.16
Visual Cortex	2.26	0.39	0.12
Caudate	2.85	0.32	0.10
Putamen / Globus Pallidus	2.80	0.37	0.12
Thalamus	1.74	0.49	0.15
Brainstem	1.87	0.72	0.23

10 patients second study

Table # 11. t-tests for independent samples with unequal variances

Region	# of Patients	Mean FDG counts	SD	Uneq.Var t-value	df	2-Tail Sig
R-thalamus	24	127538.3800	57865.650			
R-hemisphere	24	12072207.858	4224154.26	-13.85	23.01	0.000
L-thalamus	24	115702.5017	54238.458			
L-hemisphere	24	12284157.671	4329814.36	-13.77	23.01	0.000
R-thalamus	24	127538.3800	57865.650			
R-basal ganglia	24	59887.8151	23080.803	5.32	30.14	0.000
L-thalamus	24	115702.5017	54238.458			
L-basal ganglia	24	51732.9761	18464.011	5.47	28.26	0.000

The mean visual grade of the thalamus in 24 subjects was 1.87. Both paired and independent t-tests resulted in significant differences between averaged counts of the thalamus and basal ganglia, and averaged counts between the thalamus and each hemisphere and whole brain. In figure 3, the glucose uptake in the thalamus of a normal subject (image a) is compared with decreased thalamic metabolic activity in two patients with NF_1 (images b & c).

This series attempted to give insight into the utility and value of FDG PET imaging in NF_1 patients with central nervous system tumors. Optic pathway and brainstem tumors may pose a difficult problem because these tumors are in general histologically benign but can occasionally have an aggressive course. Our study did not have a long enough follow-up, nor did enough patients progress to generate patient outcome predictions based on imaging.

As a part of this study, other important PET imaging data has emerged. Areas of cortical and subcortical regions of hypometabolism have been identified, most notably in the thalamus. We find this an interesting result because the thalamus as the possible target area for neurocognitive deficits in NF_1 has been suggested previously by other investigators (Moore et al, 1996, Kaplan et al, 1996). Kaplan et al reported decreased glucose metabolism in ten FDG PET studies in the thalamus of NF_1 patients (Kaplan, 1996). Our much larger study of 35 patients corroborated Kaplan's results.

The role of the thalamus in arousal and awareness of sensory stimuli, hearing, vision, sensation and pain, motor function, mood and memory is well known. Thalamic dysfunction has been associated with aphasia), memory impairment, dementia, and alterations in mood and personality (Bogousslavsky, 1988; Gutman, 1991; Bogousslavsky, 1991; Castaigne, 1981; Fazio, 1992). These syndromes have been reported in infarctions, neoplasms, alcoholism, and lesions of stereotactic surgery and outcome after head injury. Lesions of the anterior and medial thalamus has been accompanied by memory dysfunction while major memory deficits have been reported with bilateral thalamic lesions. Visual-spatial memory may be disrupted by right thalamic lesions and verbal memory may be lost after left thalamic injury (Graff-Radford, 1984; Speedie, 1983; Speedie, 1982). The thalamus clearly plays a crucial role in central nervous system function and thalamic deregulation may provide the basis for understanding neurocognitive dysfunction in NF_1.

The underlying neuropathology for cognitive deficits in NF_1 is unknown. The coincidence of neuropsychological deficits and discrete MRI neuroimaging abnormalities (FASI) has led several investigators to question whether the two phenomena could be correlated. Early reports found no such association between FASI and cognitive dysfunction (Duffner, 1989; Ferner, 1993) but more recent studies concluded, to the contrary, that such a correlation exists (North, 1994; Joy, 1995; Denckla, 1996). North and collaborators compared multiple neuropsychological measures in NF1 subjects with and without FASIs (n=25 and 15, respectively) and found that the presence of FASIs was associated with lower scores on almost all cognitive measures examined (North, 1994). By contrast, Moore and colleagues found very few significant differences when they compared children with and without FASIs (n=29 and 35, respectively) on multiple neuropsychological tasks (Moore, 1996). While Moore et al found no correlation between simple presence or absence of FASIs, they did report that the presence of FASIs in the thalamus correlated significantly with lower performance IQ and poor performance

on motor, attentional, and memory tasks. It is interesting that Moore et al's study which included the largest sample size to date, did find a correlation between thalamic FASI and neurocognitive dysfunction. This was true despite the fact that FASI are much less common in the thalamus than other locations such as the basal ganglia.

The importance of thalamic hypometabolism on FDG PET imaging and FASI on MR imaging on neurocognitive deficits in NF1 remains unknown. Scientific evidence to date does not permit a definitive conclusion regarding the relationship of either FASIs noted on MRI or hypometabolism noted on FDG PET with neuropsychological impairments in NF1 patients. Although the total number of FASIs may not be of clinical significance, the presence of FASIs in particular neuroanatomic locations, specifically the thalamus may correlate well with neuropsychological function. Lack of consistent scientific evidence of the significance of neuroimaging findings, suggest that further functional neuroimaging may prove enlightening where mere neuroanatomic imaging has failed.

D. Conclusions from the Multi-Modality Neuroimaging Studies of NF1 Related Optic Pathway and Brainstem Gliomas

1. Conclusions from Magnetic Resonance Spectroscopy

1. NF1 patients with brainstem tumors had increased choline and decreased creatine similar to histologically benign low grade astrocytomas in non-NF patients.

2. Optic pathway tumors had increased choline, similar to the metabolite pattern seen in non-NF1 low grade astrocytomas.

3. Our MRS study suggests that NF1 related optic pathway and brainstem tumors are similar in metabolite profile to low grade astrocytomas in non-NF1 patients.

4. In the NF1 related optic pathway tumors, creatine was not decreased significantly, as was expected, and may reflect a biochemical difference in these tumors in the NF1 versus non-NF1 populations.

5. Three dimensional (3D) multivoxel proton spectroscopic imaging (^{1}H-MRSI), demonstrated increase in both choline and creatine in the thalamus. These metabolites were specifically increased in thalamic voxels that demonstrated focal areas of increased T2 signal on MRI (FASI), but were also mildly elevated in "normal -appearing" MRI regions.

6. The finding of increased choline and creatine in the thalamus supports our finding of ubiquitous hypometabolism in the thalamus on FDG PET in NF1 patients.

7. Implementation of 1H-MRSI in pediatric NF1 patients has proven to be an effective semi-quantitative measurement of metabolites with signal intensities and metabolite ratios and

as an application of an innovative imaging technique never previously investigated in pediatric patients including those with NF.

8. Finally, our results from CSI and 1H-MRSI may contribute significantly to our understanding of the neuropathology of NF1 by characterizing both tumors and regions of hypometabolism identified on PET and FASI identified on MRI.

2. Conclusions from Magnetic Resonance Perfusion Studies

Although many fewer MR perfusion studies were completed than anticipated, we found that MR perfusion techniques in NF1 patients can provide high resolution quantitative images with ease and safety. Many technical modifications were made that may be implemented in future studies. The modifications made over the study period in our quantitative MRI perfusion imaging techniques are now producing accurate, fairly non-invasive measures of blood flow to tumor and other brain regions in patients with NF1. In future studies, blood flow images may complement indices of metabolic activity obtained from FDG PET and may correlate with MRS in patients with NF1 .

3. Conclusions from 18-FDG PET

Again, this research represents the largest pediatric study utilizing FDG PET in patients with Neurofibromatosis Type 1. From this data, we conclude the following:

1. Both brainstem and optic pathway tumors were as a group metabolically inactive with few exceptions. During the time period of the proposal, we did not have sufficient patients with tumors imaged at diagnosis and again during progression which would allow us to evaluate the role of metabolic imaging as a true prognostic factor. While longer follow-up would be required to correlate FDG PET images with central nervous system tumor progression, these studies have been productive in an unexpected fashion, specifically elucidation of other brain regions.

2. We have learned much about the functional map of Neurofibromatosis Type1, both at the site of known brain tumors and in adjacent areas as well as unaffected brain regions. Of great interest, has been the hypometabolism noted in the visual cortices and temporal lobes and especially the thalamus,.

3. To our knowledge, no other central nervous system disorder has demonstrated such striking uniform imaging abnormalities in the thalamus. The pervasive hypometabolism noted in the thalamus of NF1 patients may advance our understanding of this neurologic /neurocognitive deficits of this disorder.

4. In addition, these FDG PET studies may have important implications for the role of the thalamus in other childhood neurologic diseases.

In conclusion, as a result of this research grant we were able to generate the following hypotheses to be tested in future NF1 studies stated previously in our discussion:

1. Metabolite levels in central nervous system tumors of NF_1 specifically low grade astrocytomas of the brainstem and optic pathways, may be substantially different from normal non NF_1 low grade astrocytomas and may help to explain the biochemistry and behavior of NF1 related brain tumors.

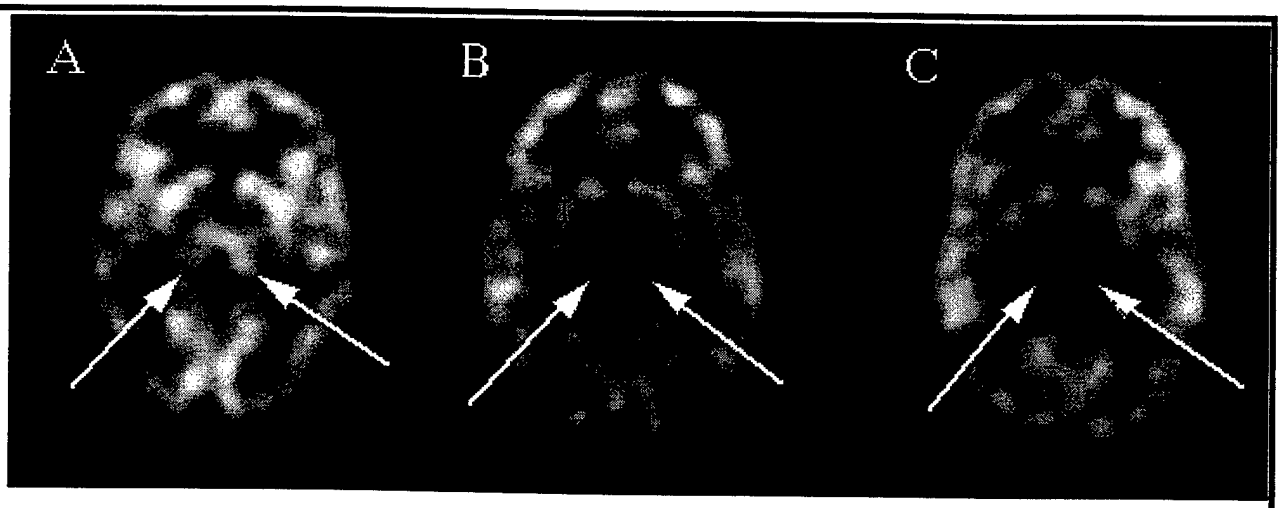
2. Specific metabolite profiles identifiable in brain tumors in NF_1 patients may allow for differentiation from surrounding focal areas of increased signal intensity (FASI) seen on T2 weighted MRI.

3. Metabolite levels in the thalamus and other brain regions of NF_1 patients, even those with normal appearing MRIs, may be substantially different than normal non NF_1 controls.

4. Decreased glucose uptake in the thalamus and other brain regions on FDG PET in NF_1 patients may correlate with abnormal metabolite levels of choline, creatine and N-acetylaspartate on 1H-MRSI.

5. Decreased glucose uptake in the thalamus and other brain regions on FDG PET in NF_1 patients may be correlated with FASI on MRI and clinical neurologic and neurocognitive dysfunction.

Figure # 2



Normal glucose uptake in the thalamus (arrows) in a control subject (image a) compared to decreased glucose uptake in the thalami (arrows) of two NF1 subjects (image b & image c)

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FDG PET CORRELATION WITH CLINICAL OUTCOME IN PATIENTS WITH NEUROFIBROMATOSIS TYPE I AND LOW GRADE ASTROCYTOMAS

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ABSTRACT

Background: Low grade astrocytomas in NF_1 patients are often clinically silent, while others continuously progress causing significant morbidity, even death. Increased glucose utilization with fluorodeoxyglucose (FDG) positron emission tomography PET has correlated well with histologic grade in gliomas in the non-NF population.

Objective: To assess the diagnostic value of FDG uptake in NF_1 related low grade astrocytomas and to compare tumor glucose uptake on PET imaging with clinical outcome.

Methods: All patients had NF_1 and tumors of the optic pathways, thalamus or brainstem. Patients were grouped into categories of "treated" versus "not treated" based on need for treatment and categories of "stable" versus "progressive" disease based on disease outcome. Glucose uptake on FDG PET studies was then correlated with the need for treatment, disease outcome and histopathologic features.

Results: Twenty-four patients with NF_1 were enrolled prospectively with a mean follow-up of 32.8 months. Fourteen patients made up the "no treatment" group and ten patients made up the "treatment" group. Thirteen of 14 patients (93%) in the "no treatment" group demonstrated no increase in tumor glucose uptake on PET consistent with histologically benign tumors. By contrast, seven of 10 patients (70%) in the "treatment" group had increased tumor glucose uptake on PET imaging consistent with high grade or malignant disease. Sixteen of 17 patients (94%) with "stable" disease had tumors without increased glucose uptake compared with seven patients (100%) with "progressive" disease whose tumors did have increased glucose uptake. Twenty-three of 24 patients (96%) had clinical outcome (progressive vs stable disease) that correlated with tumor metabolic activity on FDG PET ($p < .00005$). Three of four patients with pathologically proven low grade astrocytoma had metabolically active tumors despite benign histopathology.

Conclusions: FDG PET accurately distinguished clinically stable astrocytomas from those with progressive growth. Glucose uptake on [18] FDG PET was correlated with need for treatment and clinical outcome and was not associated with histologic grade of tumor.

Key Words: Neurofibromatosis Type I (NF_1), Low Grade Astrocytomas, Optic Pathway Tumors, Brainstem Tumors, [18] Fluorodeoxyglucose (FDG) Positron Emission Tomography (PET)

Background

Neurofibromatosis Type I (NF_1) is an autosomal dominant disorder, with a predilection for both benign and malignant tumors, especially in the central nervous system. The most common intracranial tumors are low grade astrocytomas of the optic pathways, hypothalamus and brainstem. The incidence of optic pathway tumors in NF_1 patients ranges from 1.5% in an NF I population-based survey [1] to 15% in NF_1 referral centers. [2,3] The incidence of brainstem tumors in NF I patients has not been established. While the frequency was estimated at 4% in a large NF_1 clinic population[4]; another investigator reported 21 brainstem tumors in 241 NF_1 patients who underwent neuroimaging studies[5] suggesting a higher frequency. The growth of these tumors is also unpredictable, ranging from clinically silent to rapidly progressive disease, often causing significant morbidity, and even mortality.

This variability in growth rate has impeded advances in the treatment of intracranial tumors in NF_1 patients. Periods of spontaneous growth arrest after an interval of rapid tumor growth are well described in NF_1 low grade astrocytomas. As a result, it is difficult to determine when medical intervention is appropriate for NF_1 patients with tumor progression. Furthermore, when patients with tumor progression are treated, it is difficult to determine if the tumor stability is attributable to the therapeutic intervention or represents a spontaneous growth arrest.

Another critical problem in the treatment of NF_1-related tumors arises from the lack of a reliable, non-invasive diagnostic modality that will accurately distinguish tumors with a low growth potential from those with a high growth potential. To address this need, a neuroimaging trial was established utilizing positron emission tomography (PET) as a potentially important diagnostic and prognostic imaging technique.

Functional imaging using positron emission tomography has been increasingly exploited as a non-invasive imaging tool to assess tumor activity. Besides providing important information regarding tumor activity, PET has been useful in tumor prognosis as well as in distinguishing recurrent tumor from radiation necrosis.[6,7] Many PET studies in brain tumor patients have utilized FDG and it remains the most widely used isotope for the investigation of brain tumors[7, 6; 8]. FDG studies have concluded that high grade tumors are hypermetabolic and low grade tumors are hypometabolic [9]. PET may more accurately predict the degree of malignancy of a tumor compared with standard neuroimaging techniques (CT or MRI), and FDG PET has been shown to predict survival in patients with gliomas more accurately than either CT or MRI. [8, 9, 10]

The purpose of this study was to attempt to predict tumor growth potential in patients with NF_1 and tumors of the optic pathways, hypothalamus and brainstem and to correlate the

clinical course of their disease with the metabolic characteristics of the tumor demonstrated on FDG PET imaging.

METHODS

All patients met the diagnosis for Neurofibromatosis Type I based on the criteria established by the National Institute of Health (NIH) Consensus Development Conference in 1987 [13]. At least two of the seven following criteria had to be present: greater than six café au lait spots; axillary or inguinal freckling; a plexiform neurofibroma or two or more subcutaneous neurofibromas; a visual pathway glioma; tibial pseudoarthrosis or sphenoid wing dysplasias; two or more Lisch nodules: and a first degree relative with Neurofibromatosis Type I demonstrating the above criteria.

All patients were required to have magnetic resonance imaging (MRI) evidence of a tumor of the optic pathways, hypothalamus or brainstem that was at least 1.0 cm^2 in cross-sectional area. All patients were required to be at least 12 months of age at study entry.

All patients were studied with cranial MRI. The MRI sequences included short TR/TE (600/15), long TR/ short TE 2500-3500/22 (proton density), and long TR/ long TE 2500-3500/90-120. The matrix was generally 256 x 256 with 1-2 acquisitions. The slice thickness ranged from 2mm to 5mm with the thinner sections through the orbits and the thicker sections through the brain. The MRI scans were obtained in three planes: sagittal, axial, and coronal. Intravenous gadolinium DTPA was used in all patients undergoing MRI. MRI studies were performed at three month intervals for the 12 months of the study.

A single venous catheter was inserted into an antecubital vein of one arm for the administration of FDG. A second venous line was used initially and discontinued out of consideration for a mostly pediatric population and to insure better study participation. All patients, who required sedation, were sedated with pentobarbital at identical doses to those used in the MRI scan sedation protocol. The sedation was initiated at a minimum 40 minutes after the administration of FDG but before any PET imaging was started. FDG was administered as a bolus 30 uci/kg (25% of standard dose) because of the high sensitivity of the HEAD-PENN-PET scanner. Forty minutes after the administration of FDG, the patient was positioned into the HEAD-PENN-PET scanner. The PET scans were acquired parallel to the canthomeatal line and included the entire brain and the upper cervical spinal cord (the axial field of view for this instrument = 26cm). The total imaging time was 30 minutes, which in our experience, was tolerated well by our pediatric NF_1 patients.

All patients, who required sedation, were sedated with intravenous pentobarbitol according to the standard protocol of The Children's Hospital of Philadelphia, which does not exceed 6 mg/kg or a maximum total dose of 150 mg. Rare patients, who were difficult to sedate, were given a single intravenous dose of morphine at 0.1 mg/kg.

The Children's Hospital of Philadelphia Institutional Review Board and the U.S. Army Approved Consent Documents were signed by patients and/or parents of patients prior to participation in these studies.

Positron emission tomography studies of NF_1 brain tumors were performed using tracer amounts of [18F] fluorodeoxyglucose (FDG) to assess cerebral glucose utilization in tumor and normal brain regions of interest.

Two FDG PET studies were planned on entry to the neuroimaging trial: one at study entry and one at follow-up in one year's time. Because of anticipated patient compliance problems as a result of both the use of radiopharmaceuticals and the placement of intravenous catheters in pediatric patients, patients with only one FDG PET study were considered evaluable.

Qualitative (visual interpretation) to determine the metabolic activity of the regions of interest was undertaken. The qualitative assessment used a five point visual grading (VG) system to score tumor uptake of [18] FDG as follows: totally absent tumor uptake (VG=1); slightly less uptake in tumor than surrounding brain (VG=2); same uptake in tumor as surrounding brain (VG=3) ; slightly to moderately increased tumor uptake than surrounding brain (VG=4); and, markedly increased tumor uptake (VG=5). This method has been validated by Kim et al (Kim, 1991).

The same nuclear medicine physician visually graded all PET regions of interest in all subjects. All PET studies were graded on two separate occasions with excellent intra-rater reliability. The nuclear medicine physician was blinded to both patient identity and clinical history. Neuroimaging (MRI) was not used to identify tumor location on PET.

Patients were grouped into categories of "treatment" or "no treatment." "Treatment" patients were defined as those requiring treatment at study entry or in the follow-up period. "No treatment" patients were defined as those not requiring treatment at study entry or in the follow-up period. Treatment was defined as surgical resection, chemotherapy or radiation therapy. Diagnostic biopsy or shunt placement for hydrocephalus was not considered tumor treatment.

Patients were also grouped into categories of "stable" or "progressive " disease. The "stable" group was defined either as requiring no therapy or having no tumor progression after a single, initial therapy. The "progressive" group was defined as continued tumor progression after initial therapy.

Glucose uptake on FDG PET studies was correlated with the need for treatment, disease outcome and histopathologic features. Statistical analysis was performed using the Fisher exact test.

Results

Twenty-four patients with NF_1 were serially enrolled in the neuroimaging trial. All patients had both NF_1 and tumors of the optic pathways, thalamus or brainstem. Fifteen patients were enrolled for optic pathway tumors and nine were enrolled for brainstem or thalamic tumors. Ten of 24 patients (42%) had tumors of both the optic pathways, brainstem or thalamus.

Fourteen patients were male and ten were female. The mean age on study entry was 28 months (median age: 40 months; range: 15-273 months). The mean follow-up was 32.8 months (median follow-up: 35 months; range: 18-53 months).

Ten of 24 patients required treatment (surgery, radiation or chemotherapy in some combination), either at study entry (seven patients), or in the follow-up period (three patients) and made up the "treatment" group. Fourteen of 24 patients never required medical intervention and made up the "no treatment" group.

Three of ten patients in the "treatment" group were stable after a single therapeutic intervention. These three patients in addition to the 14 patients, who never required medical intervention, made up the "stable" disease group (Figure I). The remaining seven patients in the "treatment" group who continued to have tumor progression after initial therapy made up the "progressive" disease group (Figure I). Three of these patients died from tumor progression, and the remaining four patients in the progressive group had further visual deterioration with two patients blind. Three patients remain on treatment for tumor progression.

Nine of the ten treated patients had both clinical and radiographic disease progression with both visual deterioration and increased tumor size on T1 weighted gadolinium enhanced MRI. One patient had visual deterioration only.

The details of patient presentation, treatment and outcome are described in Table # 1.

One patient had a diagnostic biopsy at tumor presentation and three patients had a partial surgical resection at the time of tumor progression. Two patients had a pilocytic astrocytomas (WHO classification Grade I of IV) and two patients had astrocytomas (WHO classification Grade II of IV). A fifth patient had a post mortem examination that demonstrated mixed pilocytic astrocytoma and anaplastic astrocytoma (WHO classification Grade III of IV).

Four patients with optic pathway tumors were treated with oral etoposide (VP-16) $50mg/m^2$ daily x 21 days in 28 day cycles with three patients completing all 12 cycles of VP-16. Two patients treated with VP-16 had stable disease with no further visual deterioration and no tumor progression on MRI. One patient treated with VP-16 had initial improvement of vision and minimal decrease in tumor size (<25%) on MRI but then had tumor progression with a follow-up of thirteen months. The fourth patient had both visual deterioration and radiographic tumor progression after the fourth cycle of VP-16. A fifth patient with a thalamic tumor was not

compliant with VP-16 therapy and was treated with involved field radiation therapy for tumor progression.

Three patients with optic pathway tumors, treated on an experimental protocol with either interferon alpha 2a or cis-retinoic acid, had tumor progression.

Three patients with progressive optic pathway tumors were treated with Carboplatin 175mg/m^2 weekly and Vincristine 1.5mg/m^2 for ten weeks of induction followed by eight maintenance cycles of Carboplatin and Vincristine. On this combination, two patients had both visual deterioration and radiographic tumor progression, while one patient remains on therapy with stable disease. One patient with a progressive optic pathway tumor, treated with Vincristine and Actinomycin D, had both visual deterioration and radiographic evidence of tumor progression. Two patients with progressive optic pathway tumors are currently being treated with a combination of Thioguanine, Procarbazine, Lomustine and Vincristine.

All twenty-four patients had at least one FDG PET study performed and a total of 36 PET studies were completed (Table # 2). Fourteen patients had one PET study; nine patients had two PET studies; and, two patients had three PET studies. Sixteen patients had tumors with visual grades in the 1-3 range on PET imaging with no tumor having any increase in tumor glucose uptake when compared to surrounding brain. Eight patients had tumors with visual grades in the 4-5 range on PET or moderately to markedly increased tumor glucose uptake when compared to surrounding brain.

At study entry, seven of 24 patients (29%) had clinical symptoms, specifically visual loss, requiring treatment (Table # 1). Five of these seven patients continued to have further clinical deterioration after initial therapy and all five patients had tumors demonstrating increased glucose uptake on PET imaging (Cases #1,2,3,4,6). By contrast, the two patients who stabilized after initial therapy had tumors with no evidence of increased glucose uptake on PET (Cases # 10,14).

Of note, no patient with an active tumor demonstrated on the initial PET study, had tumors that became metabolically inactive on the follow-up study. By contrast, two patients with an initial unremarkable PET study had follow-up PET studies that demonstrated an increase in tumor metabolic activity at the time of clinical disease progression (Cases # 4 and # 7).

Need for treatment was correlated with tumor glucose uptake on FDG PET. Fourteen of the patients in the "no treatment" group, thirteen patients (93%) had metabolically inactive tumors (VG = 1-3). By contrast of the seven of the ten patients (70%) who made up the treatment group had metabolically active tumors (VG = 4-5). The need for tumor treatment correlated positively with the metabolic activity of the tumors on FDG PET and was statistically significant by Fisher exact test ($p \leq .0023$).

Clinical outcome was also correlated with tumor glucose uptake on PET. Seventeen patients made up the "stable disease" group while seven patients made up the "progressive disease" group. Of the 17 patients with clinically "stable disease ," 16 patients (94%) had metabolically inactive tumors (VG = 1-3). By contrast, all seven patients (100%) with "progressive disease" had metabolically active tumors (VG=4-5). Twenty-three of 24 patients (96%) had clinical outcome (progressive disease vs stable disease) that correlated with tumor metabolic activity on FDG PET and was statistically significant by Fisher exact test ($p \leq .00005$).

Finally, glucose uptake on PET imaging was compared with histology, three of four patients with pathologically proven low grade astrocytomas (WHO classification Grade I or II) had increased tumor glucose uptake. This pattern on PET imaging was more consistent with malignant or high grade tumor biology than with low grade astrocytoma (See Figure I). The fourth patient whose pathology also demonstrated a low grade astrocytoma did not have a PET study at the time of tumor progression. A fifth patient with a pathologically proven anaplastic astrocytoma on post mortem examination had increased tumor glucose uptake on PET which was consistent with both the histology and the clinical behavior of the tumor predicted by PET. While no statistical conclusion could be reached with such a small sample size, tumor glucose uptake on FDG PET did compare more favorably with tumor biology than histopathologic features.

Discussion

Controversies about low grade astrocytomas, especially those of the visual pathways abound from their tumor biology to their radiographic presentation to medical management and even to appropriate terminology for these tumors. In fact, optic pathway tumors are not recognized as a separate pathological entity by either the WHO classification of central nervous system tumors or in standard textbooks of central nervous system pathology. [14,15,16] The name pilocytic astrocytoma of the optic pathways [17] has been commonly accepted but other issues related to these complex tumors are not so easily resolved.

One of the controversies, which is critical to the treating physician, centers around the appropriate medical management of NF_1-related low grade astrocytomas of the optic pathway, brainstem, and other brain regions. It would be critically important to have a reliable, non-invasive imaging technique that would help to discriminate between tumors with an indolent course, permitting conservative management, from tumors with a more aggressive course, which may require multiple therapeutic interventions. Therefore, the purpose of this imaging trial, was to begin to determine if FDG PET will better characterize NF_1-related tumors by helping to define their elusive tumor biology.

In our NF study population, need for treatment and clinical outcome correlated remarkably well with tumor metabolic activity on FDG PET. Not only did glucose uptake on PET correlate positively with treatment and outcome, but there was a suggestion that it may correlate better with tumor biology than histopathologic features. Despite the reportedly indolent nature of

these tumors, seven patients in our study population, had progressive disease upon entry to the trial. Five of the seven patients had poor clinical outcomes: two patients were blind; one patient had minimal vision in one eye; and two patients died from relentless disease progression. All five patients had metabolically active tumors on PET, the pattern typically seen in high grade gliomas. Seven patients stabilized with the initial medical therapy and two patients had metabolically inactive tumors on FDG PET, the pattern characteristic of low grade gliomas. In this admittedly small patient sample, functional imaging with FDG PET better reflected tumor biology than histopathologic features. When these classically "benign" low grade astrocytomas behaved in a clinically aggressive manner, these tumors demonstrated the metabolic activity characteristic of high grade or malignant tumors, a potentially important finding.

As a result of this experience, several question have yet to be answered. Could the poor clinical outcome in the treated patients be predicted based on their metabolic activity on PET? Was the more benign course of the patients that stabilized after initial therapy, the result of spontaneous growth arrest, or our medical intervention, and was this course predictable by PET? Did medical intervention affect the course of the disease in any of the seven treated patients or did the tumor biology in these NF_1 patients simply run its course?

In this trial, we reported a positive correlation between stable tumors and decreased glucose uptake and progressive tumors and increased glucose uptake. While the tumor's anatomy, in all cases, was well delineated with MRI, the simple knowledge of the tumor's existence gave no clues to the tumor's biology. Functional imaging with FDG PET augmented classic neuroanatomic imaging in our study population.

We also reported a high concurrence of both optic pathway and brainstem tumors with 42% of our study population having multiple glial tumors. It was interesting to note that the majority of patients with multiple glial tumors required medical intervention. Bilaniuk from our institution, previously suggested that there may be an NF_1 phenotype in which multiple glial tumors are expressed (Bilaniuk, 1997[18]). Our experience with multiple glial tumors in this trial suggests that this phenotype may be predictive of more aggressive disease and warrants further investigation.

Listernick et al have suggested that NF_1-related optic pathway tumors rarely progress once the tumors have come to medical attention.[19, 20] This contrasts with our study population in which several patients had continued disease progression despite the best available standard therapies both with single agents and combined modalities. Our sample size was too small with too few treated patients, to permit further observations. In addition, while it is true that the emergence of an optic pathway tumor after six years of age is extremely rare,[21] tumor progression can occur at an older age, as was demonstrated by two subjects in our study.

The clinical situation for the neuro-oncologist that is particularly difficult to resolve, is the patient with clinical deterioration following therapy, in the setting of stable neuroanatomic findings by neuroimaging. This is analogous to the dilemma, the physician has when confronted with the young child with NF_1 and an optic pathway tumor complicated by a very limited, possibly useless visual exam, but in the setting of a stable MRI. Since young children are the most difficult to evaluate, and are at the greatest risk for tumor progression, FDG PET may provide a needed diagnostic tool.

PET has become increasingly employed to assess tumor activity, predict disease outcomes and differentiate tumor from the neuropathologic changes of radiation therapy.[8,6,9,7] Clearly PET has demonstrated prognostic value with respect to clinical response and survival. It is likely that in NF brain tumor patients, FDG PET might influence patient management by providing more objective criteria for continuing or changing a specific strategy. Future studies should also explore the use of PET in the study of low grade astrocytomas in the non-NF pediatric population.

The significant results of our clinical trial, combined with the demonstrated utility of PET in brain tumor patients, warrants further collaborative trials evaluating FDG PET in NF_1 patients with low grade astrocytomas.

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FIGURE LEGEND

Case # 3 A three year old girl with NF_1 and progressive optic pathway tumor.

FIGURE I

(A) T1 gadolinium enhanced axial MRI image demonstrates tumor in the left optic radiations.

(B) Follow-up MRI (1 year later) demonstrates tumor progression at the same site.

(C) Initial FDG PET image did not demonstrate any region of increased glucose uptake.

(D) Follow-up FDG PET image (1 year later) demonstrates increased glucose uptake in the tumor in the left optic radiations.

(E) Pathologic specimen demonstrates areas of dense and loose architecture with Rosental fibers and occasional granular bodies consistent with a pilocytic astrocytoma..

Case # 4 A seventeen year old boy with an optic pathway tumor was treated with involved field radiation therapy and developed a presumed radiation induced secondmalignancy.

FIGURE II

(A) T1 gadolinium enhanced axial MRI image demonstrates tumor in the left thalamus.

(B) Follow-up MRI image (2 years later) demonstrates tumor progression at same site.

(C) Initial FDG PET image does not demonstrate any regions of increased glucose uptake.

(D) Follow-up FDG PET image (2 years later) demonstrates increased glucose uptake in the tumor in the left thalamus.

(E)(F) Post-mortem pathologic specimens demonstrate both regions of pilocytic astrocytoma (E) and regions of anaplastic astrocytoma (F).

TABLE # 1: Presentation and Treatment of NF I Patients with Tumors of the Optic Pathways, Thalamus or Brainstem Evaluated on the Neuroimaging Trial

PT#	SEX	AGE mos	HX	PE	MRI	TX	Outcome
1	F	18	↑Irritability ↑ Lethargy	(R)APD (R)Proptosis	Hydrocephalus/ Diffuse OPT	Bilateral VP shunts Observation	Progression
		23	↓ Vision	↓ Vision	Stable	VP 16 (12 cycles)	Vision better MRI ↓ tumor
		48	↓ Vision	↓ Vision	↓Tumor	VCR/CBCDA (Induction only)	Blind
		53	Blind	Blind	↑Tumor	TPCV begun	On therapy
2	M	247	↑ HA	Papilledema (L) drift	Hydrocephalus/ Bithalamic/ tectal tumor	Biopsy Observation	Progression
		253	↑ HA ↑ Lethargy	N/A	↑Tumor	VP16 (Not compliant)	Neuro exam worse MRI- ↑ tumor
		259	Somnolence	Somnolence Multiple cranial neuropathies (L) Hemiparesis	↑ Tumor	XRT (54 GY)	Death
3	F	35	↓ Vision	Disc pallor ↓ vision	Hydrocephalus/ Diffuse OPT	VP shunt/ Observation	Progression
		40	Stable	Stable	↑ Tumor	CRA (3cycles)	MRI ↓ Tumor
		43	Stable	Stable	↑ Tumor	VCR/CBCDA (Induction + 2 cycles of maintenance)	↓ Vision
		49	↓ Vision	↓ Vision	↑ Tumor	VP 16 (4 cycles)	↓ Vision MRI:↑ Tumor
		54	↓ Vision	↓ Vision	↑ Tumor	Partial resection VCR/Actimoycin (3 cycles)	↓Vision MRI: ↑Tumor
		72	↓ Vision	↓ Vision CF OD LP OS	↑ Tumor	TPCV begun	On therapy
4	M	196	PMH = OPT: Tx-XRT 5580 cGy/VP shunt Developed Somnolence, Dysarthria,	Multiple cranial neuropathies, (L) hemiparesis	Diffuse OPT New thalamic/ brainstem tumor	Supportive Care	Death

5	M	78 N/A	Blind, diffuse PN Progressive neurological	Blind Multiple Cranial neuropathies Quadraparesis N/A	Diffuse OPT Large head/neck PN None	IFN x 12 cycles Supportive Care	Progression Death
6	M	273 298	↓ Vision Blind	CF OU Blind	↑ Tumor ↑ Tumor Hydrocephalus	IFN x 2 cycles (patient non-compliant) Surgical resection VP shunt	Stable On therapy
7	M	37 46	Stable ↓ Vision	↓ Vision (?) ↓ Vision	OPT ↑Tumor Hydrocephalus	VP/16 VP shunt VCR/CBCDA Induction/2 cycles of maintenance	Progression On therapy
8	M	42	↓ Vision Worsening behavior, Seizures	↓ Vision PDD-like behavior	Diffuse OPT	Observation	Stable
9	F	94	Headache, Diplopia	Papilledema (R) VI and VII nerve palsies	Hydrocephalus, Diffuse BST	VP shunt	Stable
10	F	61	↓ Vision	↓ Vision Head/neck PN	Diffuse OPT	VP 16 (12 cycles)	Stable
11	M	71	No complaints	Stable Baseline	OPT	Observation	Stable
12	F	167	No complaints Hx:OPT Tx: XRT BST Tx: Surgery ? VP Shunt	Stable Baseline	Diffuse OPT BST	Observation	Stable
13	M	95	No complaints	Stable Baseline	BST	Observation	Stable
14	F	22	↓ Vision	↓ Vision	↑ OPT	VP 16 (12	Stable
15	F	68	No complaints Hx: OPT	Stable Baseline	Diffuse OPT	Observation	Stable
16	M	57	No complaints Hx: OPT, BST	Stable Baseline	OPT, BST	Observation	Stable
17	M	91	No complaints Hx: OPT	Stable Baseline	OPT	Observation	Stable

18	M	45	No complaints Hx: OPT	Stable Baseline	OPT	Observation	Stable
19	F	15	No complaints Hx: OPT	Stable Baseline	OPT	Observation	Stable
20	M	33	No complaints Hx: OPT	Stable Baseline	OPT	Observation	Stable
21	M	22	No complaints Hx: OPT	Stable Baseline	OPT	Observation	Stable
22	F	126	No complaints Hx: BST	Stable Baseline	BST	Observation	Stable
23	M	88	No complaints Hx: BST	Stable Baseline	BST	Observation	Stable
24	F	136	BST Swallowing dysfunction, Hiccups	No gag	↑ Tumor	STR, VP Shunt LGA	Stable

Legend

M = Male
F = Female
P T = Patient
# = Number
Mos. = Month

Hx = History
PE = Physical Exam
(R) = Right
(L) = Left

↑ = Increased
↓ = Decreased
HA = headache
OPT = Optic Pathway Tumor
BST = Brainstem Tumor

PN = Plexiform Neurofibroma
XRT = Radiation Therapy
CF = Counts Fingers Vision
NLP = No Light Perception
VP = Ventriculoperitoneal

APD = Afferent pupillary defect
IFN = Interferon alpha 2 a
VP 16 = Etoposide
CRA = Cis-Retinoic Acid
TPCV = Thioguanine, Procarbazine, CCNU, Vincristine
CBCDA = Carboplatinum
MRI = Magnetic Resonance Imaging
LGA = Low Grade Astrocytoma
N/A = Not available (missing data)
STR = Sub-total Partial Tumor Resection

Table # 2: Visual Grade of Tumor on FDG PET compared with clinical outcome and histopathologic features in patients with NF_1-related Low Grade Astrocytomas

PT#	Primary Tumor Type Evaluated	1st PET Study Tumor Location Visual Grade	2nd PET Study Tumor Location Visual Grade	3rd PET Study Tumor Visual Grade	Pathology (If any)	Clinical disease: PD vs SD	Follow-up (Mos)
1 *	OPT†	(L) OC = 4 (L) TE = 4				PD	35 mos.
2 *	Thalamus	TH = 5			LGA	PD	30 mos. Dead
3*	OPT†	(R) PA/OC = 4	(R) PA/OC = 4 (L) TE = 4		LGA	PD	37 mos.
4*	Thalamus/ BST‡	H/C = 2	H/C = 2	TH = 5	AA/LGA ■	PD	18 mos. Dead
5	OPT	(R) PA/OC = 3-4	(R) PA/OC = 4			PD	23 mos. Dead
6*	OPT†	(L) TE/PA = 4			LGA	PD	39 mos
7	OPT†	H/C = 1-2	H/C = 2	TH = 4		PD	27 mos
8	OPT†	H/C = 4-5 (R)TE = 3-4 (L) TE = 5	H/C = 4-5 (R)TE = 4 (L) TE = 5			SD	48 mos
9	BST	BST = 1-2	BST = 2			SD	53 mos
10*	OPT	H/C = 1	H/C = 1			SD	53 mos
11	OPT	H/C = 2	H/C = 1			SD	45 mos
12	BST‡	BST = 3	BST = 3			SD	39 mos
13	BST	BST = 1-2	BST = 2			SD	38 mos
14 *	OPT	H/C = 1				SD	29 mos
15	OPT†	H/C = 1				SD	29 mos
16	BST‡	BST = 1				SD	47 mos
17	OPT	H/C = 1-2				SD	34 mos
18	OPT	H/C = 1-2				SD	21 mos
19	OPT	H/C = 1				SD	21 mos
20	OPT	H/C = 1-2				SD	24 mos
21	BST	BST = 2				SD	21 mos
22	OPT	H/C = 1				SD	20 mos

23	BST	BST = 2				SD	21 mos
24	BST ‡	BST = 2			LGA	SD	37 mos

Table Summary: Fifteen patients had OPTs. Nine patients had BSTs. Sixteen patients had visual grades in the 1-3 range on PET. Eight patients had visual grades in the 4-5 range on PET.

Table Legend:

OPT = Optic Pathway Tumor	LGA = Low Grade Astrocytoma	TH = Thalamus
BST = Brainstem Tumor	AA = Anaplastic Astrocytoma	H/C = Hypothalamus/Chiasm
P.D. = Progressive Disease	(L) = Left	TE = Temporal Region
S.D. = Stable Disease	(R) = Right	PA = Parietal Region

Visual Grading (VG) System for FDG Tumor Uptake:
VG = 1: Totally Absent Tumor Uptake than surrounding brain.
VG = 2: Slightly less Tumor Uptake than surrounding brain
VG = 3: Same tumor uptake than surrounding brain
VG = 4: Slightly to moderately increased Tumor Uptake than surrounding brain
VG = 5: Markedly increased tumor uptake than surrounding brain

*= Progressive disease on study entry: Case # 1, 2, 3, 4, 6, 10, 14.
† = On study for OPT with second tumor (BST): Case # 1, 3, 6, 7, 8, 15.
‡ = On study for BST with second tumor (OPT): Case # 4,12, 16, 24.
■ = Post Mortem examination: Case # 4.

A Randomized Non-Comparative Phase II Trial of Cis Retinoic Acid or Interferon α2 a in the Treatment of Plexiform Neurofibroma in Patients with Neurofibromatosis Type I

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Abstract

Background: Almost 50% of NF_1 patients will have at least one significant plexiform neurofibroma (PN). Of those not amenable to surgery, there is no effective medical management. Nine institutions participated in a randomized non-comparative phase II trial of CRA agent and IFN to identify agents which have efficacy in PN.

Methods: All patients had ≥ 1 progressive plexiform neurofibroma, measurable clinically or by imaging (MRI or CT). Patients were randomized to CRA or IFN. Patients were treated with CRA 60 mg/m^2/day orally for 21 days with a 7 day rest. The dose was increased by 20 mg/m^2/day monthly to a dose of 140 mg/m^2/day if tolerated. Patients randomized to IFN α-2a were treated with 1,000,000 IU/m^2/day by subcutaneous injection daily. The daily dose was escalated by 500,000 IU/m^2/day every 2 weeks up to 4,000,000 IU/m^2/day if the patient did not develop myalgia/arthralgias requiring analgesia.

Results: Sixty-three patients were enrolled and fifty-six patients were evaluable for response and toxicity having completed at least three cycles (median age 11 years. Toxicity to CRA was limited to chelitis, dry skin, rare headaches and elevation in cholestorol. Toxicity to IFN was limited to mild elevations in liver enzymes and rare leukopenia. No patient underwent an objective response.

Of the 29 patients treated with CRA, two patients had evidence of tumor shrinkage (< 25%) by physical exam and of 27 patients on IFN, three patients had tumor shrinkage (< 25%) by physical exam with a median follow-up of 18 months. Of 29 patients treated with CRA, three patients had tumor progression on MR/CT imaging and one patient had tumor progression by physical examination. Of 27 patients on IFN, no patient had evidence of tumor response on MR/CT imaging, but three patients had evidence of tumor shrinkage by physical exam. Five of 27 patients on IFN had symptomatic improvement: one patient had resolution of bradycardia secondary to a vagal nerve tumor, one patient had resolution of orthopnea, and three patients had relief of pain. Overall 4 patients had stable disease and five had evidence of minimal tumor shrinkage by physical examination.

An unexpected result was the frequency of tumor stabilization in treated patients, considering that all tumors were progressing at the time of study entry. A number of patients reported that treatment resulted in the longest period of tumor stabilization and the longest interval between surgeries. The rates of progression in both treatment arms were less than anticipated for this population based on patients in the retrospective CHOP study of surgical intervention (Needle et al, 1997).

Conclusions: **(1)** The treatment of malignant solid tumors is a poor model for a trial of PN in NF 1. **(2)** The number of treated patients who progressed after initiation of treatment was less than expected, judging from retrospective data on tumor progression following surgery in a similar population. **(3)** Tumor measurement was difficult due to the nature of the tumor and may have contributed to a discrepancy between clinical activity and radiographic response. **(4)** Toxicity was modest, and reversible, but a number of patients withdrew due to discomfort, on average at the six month interval. **(5)** Natural history data regarding the rate of progression of PN in the untreated state is lacking. There are few studies regarding the prognostic factors that predict progression beyond the retrospective experience at CHOP. In a single arm phase II study, the investigator needs to know what the expected outcome would be if the patient were not to be treated. A design that includes an untreated control group is the only acceptable solution.

Background

Neurofibromas are both the defining feature of Neurofibromatosis (NF1) and the cause of much of the medical morbidity and even mortality from this disorder. Neurofibromas are benign tumors consisting of fibroblasts, perineurial cells, axons (Fisher, 1968;[1] Hirose, 1986;[2] Peltonen, 1988;[3] Stefannson, 1982)[4], mast cells (Riccardi, 1990;[5] Nurnberger, 1994;[6] Carr, 1993;[7] Penfield, 1932; [8]Wagenner, 1966;[9] Schober, 1992)[10], and extracellular matrix (Penfield, 1932)[8]. Plexiform neurofibromas are growths that arise along the length of a nerve and may involve multiple nerve fascicles occurring in approximately 25% of patients with NF 1 (Riccardi 1992)[11]. It is the plexiform neurofibroma which is responsible for the major medical burden, compromising vital structures such as the trachea or spinal cord.

The only available definitive treatment of PNs has been surgery, but most lesions are not amenable to surgical extirpation. Only patients with small, well circumscribed, accessible PNs, are likely to benefit from surgery (Donner, 1994;[12] Seppälä, 1995)[13]. In a retrospective pediatric study, Needle et al described a large population of NF patients for whom surgery was not adequate as the sole medical intervention (Needle, 1997)[14]. Patients at greatest risk (>65%) for tumor progression were those with tumors of the face, neck or trunk, incompletely excised tumors, and tumors in younger age patients (age < 10 years).

The standard alternative to tumor surgery has been radiation and chemotherapy but there are no such trials in patients with plexiform neurofibromas to suggest whether such modalities are safe or effective. In fact, Maris et al reported five children with NF1 who developed myloid leukemia following treatment for another cancer type (Maris, 1997)[15]. Most concerning was the short latency to the development of a second malignant neoplasm (SMN) and the higher rate of development of SMNs than the general oncology population.. The use of cytotoxic chemotherapy in patients with PN has been limited to anecdotal experience with no published studies.

To address the needs of patients with plexiform neurofibroma who were not amenable to surgery, the Neurofibromatosis Clinical Consortium Trial (NFCC) was organized with a major focus being the conduct of clinical trials for the treatment of PNs. The first clinical treatment trial of PN consisted of a randomized non-comparative phase II trial of 13 cis-retinoic acid (CRA) and interferon α-2a (IFN). The rationale for the use of CRA was based on data suggesting that CRA has the potential to alter the splicing pattern of the NF1 gene transcript (Nishi and Saya 1991)[16]. Retinoids have also been investigated as differentiating agents in cancer . The rationale for the use of IFN came from the work of Judah Folkman in anti-angiogenesis(Folkman 1990)[17]. In this model, all tumors, need a growing vascular supply to support tumor growth; therefore, any agent that will interfere with angiogenesis should inhibit or reverse tumor progression. Because IFN is an agent with such properties, it has been used in hemangiomas of infancy (Ezekowitz, 1992;[18] 1994;[19] 1995)[20].

Methods:

All patients met the diagnosis for NF1 based on NIH Consensus Committee Criteria (NIH, 1988)[21]. At least two of the seven following criteria had to be present: greater than six café au lait spots; axillary or inguinal freckling; a plexiform neurofibroma or two subcutaneous neurofibromas; an optic nerve glioma; characteristic skeletal abnormalities i.e, skeletal dysplasias or a first degree relative with NF1 demonstrating the above criteria. Patients were required to have at least one progressive plexiform neurofibroma that was measurable by either direct observation or cross-sectional imaging (MRI or CT).

Consent forms from the Institutional Review Board of each hospital and U.S. Army approved consent documents were signed by patients and/or parents or guardians of patients prior to participation in these studies.

Patients were then randomized to receive either CRA or IFN. Randomization to either CRA or IFN was performed by The Children's Hospital of Philadelphia.

Patients randomized to CRA were treated with 60 mg/m^2/day orally for 21 days followed by 7 days of rest. The daily dose of CRA was increased by 20 mg/m^2/day every 4 weeks up to a maximal dose 140 mg/m^2/day if tolerated. The development of grade III or IV toxicity during any cycle with CRA resulted in a dose reduction of 20 mg/m^2/day. This regimen was repeated for 13 cycles or 1 year or until there was objective tumor progression. Patients randomized to IFN α-2a were treated with 1,000,000 IU/m^2/day by subcutaneous injection daily. The daily dose was escalated by 500,000 IU/m^2/day every 2 weeks up to 4,000,000 IU/m^2/day if the patient did not develop myalgia/arthralgias requiring analgesia. Development of grade III or IV toxicity during any cycle resulted in a dose reduction of 500,000/m^2/day.

Patients with PNs had both direct measurements (tape measure) and neuroimaging evaluation (MRI or CT) every three months when possible. Clinical response was based on standard oncology phase II response criteria. A complete response (CR) = total resolution of all lesions; a partial response (PR) > 50% decrease in the sum of the greatest perpendicular diameter of measurable lesions; a minor response (MR) = a 25% to 50% decrease in the sum of the greatest perpendicular diameter of measurable lesions; stable disease (SD) $\geq$ a 25% decrease in measurable lesion progression; and progressive disease (PD) $\geq$ 25% increase in the size of any measurable lesion or the development of new lesions.

The evaluation at the start of the study for the length of the study included history, physical and the following laboratory studies: CBC, differential platelets, electrolytes, BUN, creatinine, glucose, ALT, AST, bilirubin, urinalysis and B-HCG for all women past menarche. For patients on CRA, a lipid profile and amylase were also required at the start of each cycle.

Results:

Sixty-three patients with NF1 and a progressive plexiform neurofibroma were enrolled. Twenty-eight patients were male and 35 were female. Fifty-six patients were evaluable for response and toxicity. Seven patients were not evaluable because four patients started treatment despite signing informed consent and three completed less than two cycles. The age range was 3 months to 61 years with a median age of 11 years and mean age of 54 years.

Thirty-five patients had PNs of the head and neck (Figure # 1), 9 patients had PNs of the chest and spine, 8 patients had PNs of the viscera and 4 patients had PNs of the extremities (Table # 1). Of the evaluable fifty-six patients, twenty-nine patients were randomized to CRA and 27 patients were randomized to IFN. Randomized to CRA were 19 patients with PNs of the head and neck, 3 patients with PNs of the chest and spine, 4 patients with PNs of the viscera and 1 patient with a PN of the extremities. Randomized to IFN were 16 patients with PNs of the head and neck, 6 patients with PNs of the chest and spine, 4 patients with PNs of the viscera and 3 patients with PNs of the extremities. Patients randomized to CRA completed a mean of eight cycles, while patients randomized to IFN completed a mean of nine cycles, which was not significantly different.

Toxicity was manageable with both agents. The major toxicity with CRA was chelitis and dry skin with occasional headaches and elevations in cholesteral. Four patients with CRA had grade 3 skin toxicity and one patient had grade 3 cardiac toxicity and all five withdrew from treatment. Five additional patients on CRA withdrew from therapy due to discomfort (dry eyes/dry skin) without grade 3 or 4 toxicity. For most patients on CRA, skin dryness was treatable with emollients. Toxicity to IFN was limited to occasional elevation in liver enzymes and leukopenia with seven patients experiencing grade 3 hematologic toxicity (leukopenia) and one patient experiencing grade 4 hepatic toxicity. A total of nine patients withdrew from interferon because of toxicity or the pain associated with daily subcutaneous injection.

The Neurofibromatosis Clinical Consortium (NFCC) was designed along the lines of a standard phase II oncology new agent trial requiring tumor shrinkage $\geq$ 50% for an objective response. By the criteria defined in the protocol, there were no patients on either agent whose plexiform neurofibroma exhibited an objective response to treatment. No patient had radiographic evidence of tumor shrinkage and few patients had evidence of tumor shrinkage by direct physical exam.

Of the twenty-five of 29 patients treated with CRA, twenty-five (86%) had stable disease months. No patient had evidence of decreased tumor on MR/CT imaging. Only two patients with stable disease had evidence of tumor shrinkage (< 25%) by physical examination (direct measurement of the superficial component of their tumors) on MR/CT imaging at a median follow-up of 18 months. Four patients had evidence of tu-

mor progression. Three patients had evidence of tumor progression on MR/CT imaging and one patient had evidence of tumor progression by physical examination

Of 27 patients treated with IFN, twenty-six patients (96%) had stable disease at a median follow-up of 18 months. However, three patients had evidence of tumor shrinkage (< 25%) by physical examination (direct measurement of the superficial component of their tumors) at a median follow-up of 18 months but no patient had evidence of tumor response on MR/CT imaging.

Of the 27 patients on IFN, five had evidence of symptomatic improvement. Three patients had relief of pain. One patient had resolution of bradycardia secondary to a vagal nerve tumor and one patient had resolution of orthopnea, and Of the three patients with pain relief, the first patient no longer required analgesia, the second patient confined to a wheelchair was able to ambulate with less pain, and a third patient had initial pain relief which then recurred.

Overall 96% of the patients on IFN had stable disease or minimal decrease in tumor size compared with 86% of the patients on CRA ($p = 0.58$) (Table # 2). Five of 56 patients (8.9%) had evidence of symptom improvement and five of 56 patients (8.9%) had evidence of tumor shrinkage with a total of 10 of 56 patients (17.8%) demonstrating some minimal clinical improvement on either CRA or IFN. Four patients (13%) on CRA had evidence of tumor progression compared with one patient (3.7%) on ($p = 0.35$). Of interest, eight patients on IFN had evidence of either minimal tumor shrinkage or symptomatic improvement compared with only two patients on CRA which was statistically significant ($p = .038$)

The frequency of tumor stabilization was an unexpected finding particularly when considering that all tumors were progressing at the time of study entry. The rates of disease progression for both CRA and IFN was less than anticipated for this population based on patients in the retrospective CHOP study of surgical intervention (Needle et al., 1997).[14] While use of historical controls is not optimal; the surgical group is a reasonable population for comparison because both our clinical trial patients and the surgical patients had tumors that required medical intervention.

Discussion

The following six clinical observations were evident from the treatment trial of CRA and IFN in plexiform neurofibromas.

First, we found that a treatment trial design appropriate for malignant solid tumors is a poor model for a treatment trial of PN in NF 1. Unlike cancer, residual tumor in NF 1 is not necessarily fatal, and prolonged tumor control is potentially a positive outcome. A number of the patients reported the longest period of stable disease, and for some the longest interval between surgeries. A trial design in which tumor stability is a positive outcome may be a more appropriate standard.

Second, the number of treated patients who progressed after initiation of treatment was less than expected, judging from retrospective data on tumor progression following surgery in a similar population (Needle, 1997).

Third, tumor measurement was difficult due to the diffuse, interdigitating nature of the tumor and may have contributed to the discrepancy between clinical activity and radiographic response. Innovative neuroimaging techniques for assessing tumor size and shape would be critical. One such technique is magnetic resonance neurography, using T2 weighted fast spin-echo fat-suppressed imaging, with phased array surface coils to obtain high resolution images in the evaluation of patients with peripheral nerve pathology including plexiform neurofibromas.(Filler, 1996; Kuntz, 1996).

Fourth, there is insufficient data regarding the rate of progression of PN in the untreated state, and little information outside of our retrospective experience at CHOP regarding the prognostic factors that predict progression. In a single arm phase II study, the investigator needs to know what the expected outcome would be if the patient were not to be treated. In the case of patients with recurrent cancer, the expected outcome is tumor growth and death. In patients with plexiform neurofibroma, there are no solid data for patterns growth in untreated patients. The data gleaned from the surgical experience at CHOP provide for some comparison, but patient selection for surgery (over a 20 year period) was likely to be subjective and variable, and not necessarily comparable to patients who will enroll on a treatment study. The only acceptable solution is a design that includes an untreated control group.

Fifth, the degree of toxicity that led patients to withdraw from the study was much less than one would have been anticipated in a cancer study. In the case of a plexiform neurofibroma, where the threat to life is less immediate than with a malignancy, patients are less willing to tolerate toxicity. Therefore, finding appropriate empiric agents for the treatment of plexiform neurofibromas will be difficult.

Sixth, we used tumor progression as a criteria for study entry onto the treatment study. This was an attempt to identify patients at risk for further progression; however, there is no data to suggest that this is the only group at risk, or even the cohort at greatest risk, for further morbidity. The identification of prognostic factors for PN progression is essential for the identification of proper study cohorts. Any future study should ascertain data regarding risk factors from untreated cohorts, in order to aid in patient selection for future studies. Tumor stabilization from some form of medical therapy could have a major impact on management of the NF1 patient with plexiform neurofibroma. When faced with a patient with a progressive plexiform neurofibroma, who is not a suitable candidate for surgery because of tumor location, patient age, or the limited potential for gross total resection, the treating physician could use medical therapy to obviate the need for surgery or even delay surgery for better long term tumor control.

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It was this retrospective study that demonstrated the large population of NF 1 patients for whom surgery did not provide the definitive answer for the treatment for their PNs. Patients with tumors of the face, neck or trunk, younger age patients (< 10 years) and patients with incompletely excised tumors were at the greatest risk (>65%) for tumor progression and were the population most in need of medical intervention. The evidence of tumor stabilization in a similar population treated with either CRA or IFN was important since it suggests that it may be possible to halt the growth of PNs with medical therapy.

Prognostic signs in the surgical management of plexiform neurofibroma: The Children's Hospital of Philadelphia experience, 1974-1994

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Objectives: To estimate the rate of progression of plexiform neurofibroma after surgery and to identify prognostic factors that predict progression.

Study design: A retrospective review of the inpatient and outpatient records of 121 patients, who had 302 procedures on 168 tumors over a 20-year period at a single large pediatric referral center. Data on age, location, indication for surgery, and extent of resection was analyzed for prognostic significance.

Results: The overall freedom from progression was 54%. Children < 10 years old had a shorter interval of tumor control than older children ($p = 0.0004$). Tumors of the head/neck/face fared worse than tumors of the extremities ($p = 0.0003$). Less extensive resection predicted shorter interval to progression ($p < 0.0001$). Indication for surgery was not of prognostic importance. In multivariable analysis older age and location in the extremities were predictors of a better outcome.

Conclusions: Tumor progression is a serious problem for children with plexiform neurofibroma. Younger children, children with tumors of the head/neck/face, and tumors that cannot be nearly completely removed are at particular risk. These data may be useful in helping clinicians decide which patients and which tumors are most likely to benefit from surgical intervention. (J Pediatr 1997;131:678-82)

The hallmark of neurofibromatosis 1 is the propensity for neoplasia. The most common intracranial neoplasm is the optic pathway glioma. Between 15% to 20% of patients with NF1 will have enlargement involving either the optic pathway, including the optic nerves, chiasm, optic tracts, or a combination of these structures.[1] The most common peripheral neoplasm is the plexiform neurofibroma, a benign tumor of peripheral nerve.[2] Patients can have multiple lesions. Although tumors can develop throughout life, puberty and childbearing are considered by some to be the periods of greatest risk.

The management of patients with plexiform neurofibroma is not well defined. Certainly, most lesions do not require medical attention. These lesions can be large or small, but by virtue of location or the level of tolerance of the patient, often do not make an impact on the patient's well-being. For clinical purposes, neurofibromas can be grouped by location into a limited number of categories. Tumors of the face can cause disfigurement, which frequently leads to social withdrawal or to disability because of destruction of a sensory organ. This is best exemplified by tumors that infiltrate the orbit, displace the globe, and leave the patient without useful vision in the affected eye. Paraspinal tumors, frequently referred to as dumbbell lesions, can cause spinal cord compression, leading to paresis or paralysis. These lesions can cause sufficient destruction of the vertebral bodies to result in instability of the spine and secondary scoliosis. Tumors in the mediastinum can, by compressing the trachea or great vessels, cause life-threatening cardiopulmonary compromise. Tumors of the extremities can, by local destruction or venous stasis, cause significant loss of function to the point of preventing ambulation. Tumors in other locations, if they are large, painful, or tender to compression, can be a source of significant morbidity. Alteration in the character of tumors, particularly rapid growth, warrant biopsy to consider the diagnosis of neurofibrosarcoma.

CHOP	Children's Hospital of Philadelphia
NF1	Neurofibromatosis 1

Surgical intervention is the only therapy available at present because no medications can prevent or treat plexiform neurofibroma.[2] The major limitation to the surgical approach is the infiltrating nature of the tumors and the high rate of tumor regrowth. Although it is well known that regrowth is common, little is

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known about the circumstances that might favor long-term tumor control. We undertook a retrospective review of the surgical experience of Children's Hospital of Philadelphia in the hope of identifying prognostic factors that would predict the outcome of surgery for plexiform neurofibroma.

METHODS

The Neurofibromatosis Clinic at CHOP has been in operation since 1979, staffed by physicians from Genetics, Neurology, and Oncology, with consultative services provided by virtually all services in the hospital. Patients with suspicious skin findings are referred to establish the diagnosis, and other patients are referred for evaluation and treatment of specific problems. Not all patients referred from the community to a staff surgeon are evaluated in the Neurofibromatosis Clinic. By reviewing the reports in the Department of Surgical Pathology of CHOP between 1974 and 1994, 166 patients were identified with the diagnosis of plexiform neurofibroma. Of these patients 149 were found to have operative reports corresponding to the pathologic specimen. The remaining were referred for second opinion. Of these 149, 121 patients (81%) were identified who met the diagnostic criteria for NF1[3] and had been subsequently examined at CHOP or who could be reached for follow-up. The remaining 28 could not be found. From these 121 patients, the authors were able to identify procedures on 168 individual tumors or tumor regions. The total number of procedures was 302 (mean, 1.80 per tumor, range, 1 to 12). For the purpose of data analysis the 168 tumors are treated as individual events because no data exist in the literature to suggest consistent biologic behavior of multiple tumors within a single patient.[4]

In addition to the 166 patients with neurofibroma, six additional patients were identified who had neurofibrosarcoma and NF1. All six were diagnosed at the first operation. None of these patients survived. They are not included in this study cohort.

Data were collected from a number of sources. Data regarding the demographics of the patients were collected from either the hospital chart, the outpatient records of the surgical services, or the Neurofibromatosis Clinic chart. Data regarding the indication(s) for surgery and the extent of surgical excision were gathered from the operative notes. When the primary indication for surgery was cosmetic and in the case of lesions not causing pain or dysfunction, the procedures were considered elective. Other indications were dysfunction, pain, suspicion of cancer in patients known to have NF1, and diagnostic biopsy in cases in which the diagnosis of NF1 was uncertain. Data regarding location of tumor were abstracted from the patient chart. It can often be difficult to distinguish multiple tumors in a specific region from a larger infiltrating tumor. We therefore considered all procedures on a single body region (such as the mediastinum or a single extremity) as if the tumor in the region was a single tumor. For the purpose of analysis of location of tumor as a prognostic variable, tumors were assigned to three regions, head/neck/face, extremities, and trunk (including thorax, mediastinum, spine, and viscera). For the purpose of this study gross total resection was defined as complete removal of tumor, near total resection was defined as greater than 90% tumor removal, subtotal resection was defined as greater than 50% but less than 90% tumor removal, and biopsy was defined as less than 50% tumor removal. Note that the term "diagnostic biopsy" is used to denote an indication for surgery and is different from the term "biopsy" used to denote extent of resection. In all cases extent of surgical excision was determined by the operating surgeon at the time of surgery. Follow-up data regarding duration of tumor control and surgical morbidity were assessed from outpatient charts and by patient interviews in the NF clinic or by telephone. Progression was defined as the reappearance of a completely excised tumor or the regrowth of a partially excised tumor.

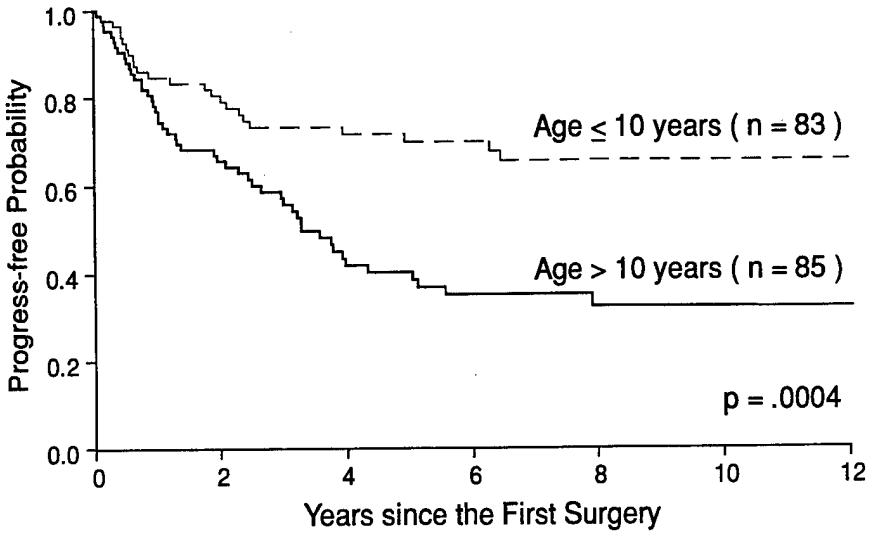

Fig. 1. Kaplan-Meier estimates of the proportion of patients without development of tumor progression in patients younger and older than the median age of 10 (n = the total number of tumors in each arm).

Statistical Methods

Kaplan-Meier curves were calculated and log rank tests were used to compare differences between progression-free survival curves based on age, location, indication, and extent of resection.[5] Cox regression models were used to explore joint predictive importance of prognostic factors for progression-free survival. Primary data analysis was conducted by using tumors as individual events, and only data concerning the first procedure were included. A confirmatory analysis was carried out using one tumor for each patient, using the patient as an independent unit of analysis, thus we did not need

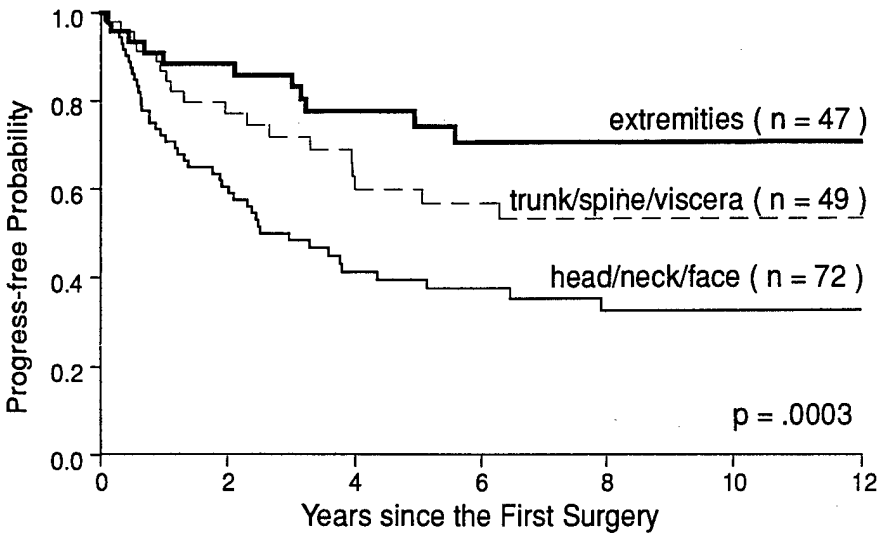

Fig. 2. Kaplan-Meier estimates of the proportion of patients without development of tumor progression based on the location of the tumor (n = the total number of tumors in each arm).

to assume lack of consistent biologic behavior of tumors within the same patient. Statistical significance was declared if the p value was ≤ 0.05. Statistical analysis was performed using Stata 4.0. (Computing Resources Center, College Station, Tex.)

RESULTS

Ninety-four of the 168 tumors (56%) did not progress after the first procedure. The remaining 74 tumors all progressed after the first procedure. The median follow-up was 6.8 years, ranging from 2 months to 24.5 years. For the purpose of identifying prognostic factors, only data concerning the first procedure were included. We analyzed these data with respect to age, indication for surgery, location, and extent of resection. There were 67 males and 54 females. The mean age at the time of the first procedure was 10 years. The most common indication for surgery was elective/cosmetic, 81 of 168 cases. The other indications, in decreasing order were dysfunction, 36 cases; diagnostic biopsy, 27 cases; pain, 16 cases; and suspected cancer, 8 cases. The most common tumor location was the head/neck/face, with 72 cases. The other locations were extremities, 47 cases; trunk, 24 cases; spine, 14 cases; and viscera, 11 cases. Trunk, spine, and viscera were combined into one category, with a total of 49 tumors. The extent of resection, as reported by the operating surgeon at the time of surgery, was gross total, 25; near total, 38; subtotal, 74; and biopsy, 31.

Each of the four recorded factors, age, location, indication, and extent of resection, was analyzed for prognostic significance. Age was categorized as children 10 years old (the median) or less and those older than 10 years of age. Fifty of 83 children 10 years of age or less had tumor progression after the first procedure (60.2%) compared with 24 of 85 children older than 10 years of age (31.2%) (Fig. 1, $p = 0.0004$, log rank). In a Cox model with age as a covariate (not grouped), older age was associated with longer interval to progression ($p < 0.0001$). Location had prognostic significance as well with tumors in the extremities doing better than tumors of the head/neck/face (Fig. 2, $p = 0.0003$, log rank).

The extent of resection was also of prognostic significance (Fig. 3). Of 25 cases of complete tumor excision, only 5 progressed (20.0%). Thirty-eight tumors had a near-total resection, and 15 (39.5%) of these tumors progressed. By comparison, 74 tumors had a subtotal resection (between 50% and 90%) with 33 (44.6%) progressing. Twenty-one of 31 (67.7%) tumors biopsied (less than 50% resection) progressed after the first procedure. These differences are statistically significant with a $p < 0.0001$ (log rank). Furthermore, for those tumors that progressed, the median time to progression was longer for patients with more extensive resection. Biopsied tumors had a median time to progression of less than 2 years compared with 5 years for subtotal resection and greater than 10 years for near total resection.

The indication for surgery was the feature with the least prognostic value. A log rank test suggests that this factor is of prognostic value only when comparing time to progression between tumors of patients in whom the primary indication for surgery is diagnostic biopsy and all other indication groups. Because a diagnostic biopsy is not an attempt at definitive therapy, this difference was not meaningful.

Cox models were fit to identify possible prognostic factors that predicted the outcome of surgery of plexiform neurofibroma jointly. Age, as a continuous variable; extent of resection; and location were prognostic for shorter interval to progression even when the variables are considered together. Age was prognostic even in the presence of other variables ($p = 0.007$). In the presence of age, location in the extremities was prognostic for longer interval to progression than other locations; however, the difference between head/neck/face and trunk was no longer significant. In the presence of age, gross total and near total resection were not different from each other in terms of prognosis, but both were different from subtotal resection and from biopsy, which were different from each other. Finally, in a model including jointly age, extent of resection (gross total, and near total together vs subtotal vs biopsy) and location (extremities vs other locations), age remained significant ($p = 0.003$) and gross and near total resection had significantly better prognosis than subtotal ($p = 0.012$) or biopsied resection ($p = 0.001$). Tumors in the extremities had significantly better prognosis than all other tumors ($p = 0.05$). Similar results were obtained when performing the confirmatory analysis in which only one tumor for each patient was considered ($n = 121$).

Permanent neurologic complications from surgery were uncommon in this pa-

tient cohort. Fourteen patients (4.6%) had permanent neurologic deficits from the total of 302 procedures. Deficits included three patients with sensory loss, two with eighth cranial nerve palsy, and one each with paraplegia and loss of bowel and bladder control, lower extremity spasticity, gait instability, vocal cord paralysis, ophthalmoplegia, wrist drop, seventh nerve palsy, and paralyzed hemidiaphragm.

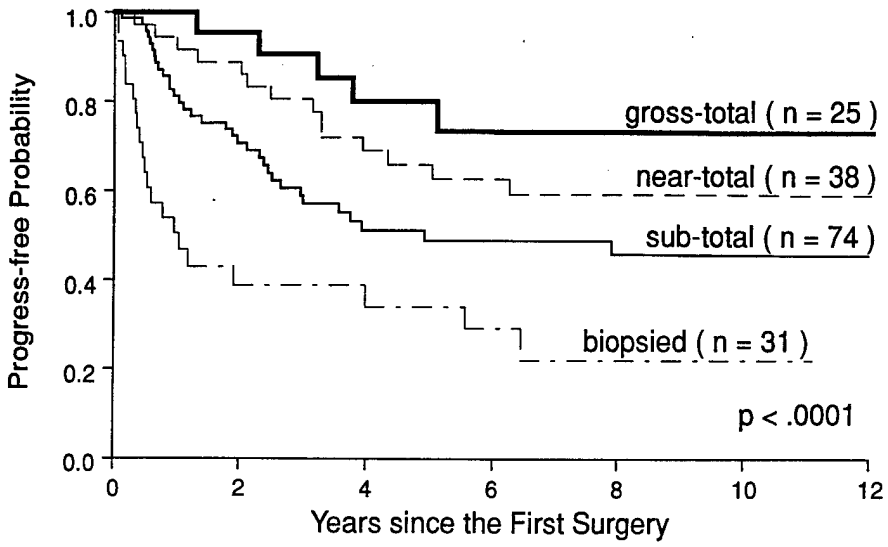

Fig. 3. Kaplan-Meier estimates of the proportion of patients without development of tumor progression based on the extent of resection as assessed by the operating surgeon (*n* = the total number of tumors in each arm).

DISCUSSION

Plexiform neurofibroma is a significant cause of morbidity and mortality in patients with NF1. Surgery remains the only viable therapy for patients in whom a tumor is causing disability or intractable pain. Additional patients have tumors that, although not causing dysfunction, are of significant concern to the patient to warrant removal for cosmetic purposes. No standardized care plans are available to guide the clinician in determining which patient will most likely benefit from surgery or in deciding the optimal time for surgery. We undertook this retrospective review of the surgical experience at a single children's hospital (CHOP) to identify prognostic factors predictive of long-term benefit from surgery.

In this analysis we considered the first surgical procedure performed on an individual tumor only. Of the 168 tumors, 94 did not progress after one surgery, the remaining 74 did progress. Age, location, and extent of surgical resection proved to be a statistically significant predictor of recurrence. Younger patients had a greater chance of tumor progression than older patients. This is most interesting in light of the widely held belief that puberty is a particularly common time of progression of plexiform neurofibroma. Perhaps the onset of puberty is the end of the period of risk defined by younger age. Alternatively, these patients may continue to have recurrences even beyond the onset of puberty because of the different biologic behavior of these tumors originating at a young age. Location was of prognostic importance as well. Tumors of the head, neck, and face were the most likely to progress, and tumors of the extremities were significantly less likely to progress. Tumors of the trunk were of intermediate risk. The difference between head/neck/face and trunk is at least partially accounted for by age. Extent of resection was of prognostic significance with greater extent of surgical resection predicting both lower risk of tumor progression and longer interval to progression.

Conceptually, there is reason to postulate interplay between location and extent of resection. Tumors of the face can be difficult to excise completely because of concerns for postoperative cosmesis. Tumors of the mediastinum and spine often insinuate between or surround vital structures preventing radical resection. Although tumors of the mediastinum were not identified as being at the highest risk of progression, that may be because the most severe tumors in this location were refused surgery for fear of operative mortality. Indication was of marginal significance, with significant differences between patients having diagnostic biopsy fairing worse than all others. This is not surprising because a diagnostic biopsy is not an attempt to provide definitive treatment. Of particular note is the relatively low rate of surgical morbidity, with only 14 reports of permanent neurologic impairment in more than 300 procedures.

Published reports on the surgical management of plexiform neurofibroma are limited. Donner and colleagues[6] described their experience at Louisiana State University over a 22-year period. Of the 288 benign peripheral nerve tumors they reported, 80 neurofibromas (6 plexiform) were in 57 patients with NF1. The average age was 28 years. This series concentrated on relief of symptoms in the perioperative period and little data are given regarding long-term tumor control. In the entire series, which includes patients with schwannoma and patients with isolated neurofibroma without NF1, the mean follow-up was 15.3 months. The high rate of success in removing tumors with little surgical morbidity is consistent with our results.

Seppälä and colleagues[7] report on 32 Finnish patients with spinal neurofibroma, 22 of whom had NF1. Five of the 22 patients with NF1 were < 18 years of age. Median follow-up was 9 years (range, 0.2 to 27.9 years). When compared with the norms from Finland, these patients had a lower than expected survival rate. Three patients had

moderate disability and one was in a vegetative state. Tumor recurrence was reported in 2 of 5 pediatric patients compared with 1 in 11 adults. The small number of patients precluded a statistical analysis.

When all the prognostic factors in this study are combined, a cohort of patients emerges that are unlikely to have long-term benefit after surgery. These patients are < 10 years old and have lesions of the head, neck, face, and trunk. Not surprisingly, many will not have a complete resection. For these patients an immediate need exists for medical therapy. A multiinstitutional consortium, The Neurofibromatosis Clinical Consortium, has been testing two biologic agents, 13-cis-retinoic acid and interferon α-2a, in a search for agents that can alter the growth patterns of these tumors. Two potential benefits from medical therapy exist. Induction of regression could render the surgically inoperable lesion completely resectable. A more modest goal would be to find an agent that is able to arrest tumor growth. This would allow a delay in therapy for the youngest patients until an age at which tumor recurrence may be less likely. It is not yet known whether arresting growth until beyond age 10 will change the long-term outcome of surgery or whether a biologic difference in tumors exists that presents and progresses at younger ages.

Plexiform neurofibroma is one of the two most common causes of substantial morbidity in children with NF1. Efforts to understand and eventually to remedy the cognitive and neoplastic manifestations of NF1 are critical. A large cooperative study of surgical management would complement the current national cooperative effort in attempting to uncover alternatives to surgery.

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